# PHYTOPATHOLOGY

## INDEX TO VOLUME 33, 1943

---

Volume 33
Number 12
December, 1943

---

1943

# PHYTOPATHOLOGY

AN INTERNATIONAL JOURNAL

OFFICIAL ORGAN OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

VOLUME XXXIII
JANUARY–DECEMBER, 1943

EDITORS

H. B. HUMPHREY — ANNIE RATHBUN-GRAVATT — G. M. ARMSTRONG

EDITOR FOR EUROPE

H. M. QUANJER

ASSOCIATE EDITORS

| | | |
|---|---|---|
| ALMA M. WATERMAN | GEORGE L. PELTIER | R. E. SMITH |
| B. L. RICHARDS | C. O. JOHNSTON | W. W. WAGENER |
| T. C. VANTERPOOL | A. G. PLAKIDAS | J. M. HAMILTON |
| S. P. DOOLITTLE | E. S. SCHULTZ | G. C. KENT |

BUSINESS MANAGEMENT

H. A. EDSON — AGNES E. MEIER — S. L. HOPPERSTEAD

---

## THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

OFFICERS

J. C. WALKER, *President*
J. J. CHRISTENSEN, *Vice-President*
C. C. ALLISON, *Secretary*
H. A. EDSON, *Treasurer*

COUNCILORS

| | | |
|---|---|---|
| L. M. HUTCHINS | H. A. RODENHISER | A. G. PLAKIDAS |
| J. G. LEACH | G. A. HUBER | O. C. BOYD |

PUBLISHED FOR THE SOCIETY
THE SCIENCE PRESS PRINTING COMPANY
LANCASTER, PENNSYLVANIA

# CONTENTS OF VOLUME 33, 1943

## NO. 1. JANUARY


Abstracts of papers accepted for presentation at the thirty-fourth annual meeting of the society, New York, N. Y., December 28 to 31, 1942 ..... 1
Peach wart. EARLE C. BLODGETT ..... 21
Fusiform rust control in forest-tree nurseries. BAILEY STEETH ..... 33
The calorific value and chemical composition of decayed cordwood. M. T. HILBORN AND F. H. STEINMETZ ..... 45
The effect of cotton seed dusting on emergence of seedlings in soil infested with Rhizoctonia. W. WINFIELD RAY ..... 51
Sulphur and copper sprays in relation to apple-tree growth and yield. H. W. THURSTON, JR., AND H. N. WORTHLEY ..... 56
Pathogenesis of Ditylenchus dipsaci in seedlings of Allium cepa. A. G. NEWHALL ..... 61
Physiologic specialization in Cercospora oryzae. T. C. RYKER ..... 70
Phytopathological Notes ..... 75
- A simple technique for isolating spores of various fungi from exposed slides in aerobiological work. W. J. MARTIN
- Distribution of Beauveria bassiana on elm insects in the United States. PAUL V. MOOK AND C. O. WOLFENBARGER
- Effect of cyanide on synthesis of ring-spot and mosaic viruses in tobacco. MARK W. WOODS
- Erwinia carotovora, the cause of a soft rot in orchids, Cattleya sp. DONALD P. LIMBER AND BERNARD A. FRIEDMAN
- A leaf-spot of Hibiscus sp. induced by Phytomonas syringae. CLAYTON O. SMITH


## NO. 2. FEBRUARY


Bacteriological sterility of tissues derived from secondary crown-gall tumors. ARMIN C. BRAUN AND PHILIP R. WHITE ..... 85
Peronospora tabacina Adam, the organism causing blue mold (downy mildew) disease of tobacco. EDWARD E. CLAYTON AND JOHN A. STEVENSON ..... 101
Cercospora blight of carrot. H. REX THOMAS ..... 114
The presence of a toxin in tomato wilt. DAVID GOTTLIEB ..... 126
Stamen blight of blackberries. S. M. ZELLER AND ALVIN J. BRAUN ..... 136
Mycorhizae associated with some Colorado flora. W. D. THOMAS, JR. ..... 144
Lightning injury to cotton. A. L. SMITH ..... 150
Decline disease of raspberry. S. M. ZELLER AND A. J. BRAUN ..... 156
Some effects of sand and nutrient supply on a physiological leaf spot of cantaloupe. E. C. PIERCE AND D. L. STODDARD ..... 162
Phytopathological Notes ..... 165
- An American oat disease found in Western Anatolia. H. BREMER
- Peach-suture spot. E. M. HILDEBRAND
- Additional records of violet scab. ANNA E. JENKINS
- Physiologic races in Urocystis tritici. C. S. HOLTON AND A. G. JOHNSON
- Cultural differences among single basidiospore isolates of Rhizoctonia solani. BEATRICE EXNER AND S. J. P. CHILTON


## NO. 3. MARCH


Increase of pathogenicity in tomato-wilt Fusarium. FREDERICK L. WELLMAN ..... 175
Occurrence, identification, and species validity of the barley loose smuts, Ustilago nuda, U. nigra, and U. medians. V. F. TAPKE ..... 194
An outbreak of plantago virus in burley tobacco. W. D. VALLEAU AND E. M. JOHNSON ..... 210
The zonate leaf spot, a new disease of sorghum. D. C. BAIN AND C. W. EDGERTON ..... 220
Another hyphomycetous fungus parasitic on Pythium oospores. CHARLES DRECHSLER ..... 227
Seedling blight and root rot of grasses in Minnesota. E. A. ANDREWS ..... 234
The effect of photoperiodism on the development of bunt in two spring wheats. H. A. RODENHISER AND J. W. TAYLOR ..... 240
The role of packing methods in the increase of anthracnose of honeydew melon fruits. W. A. KREUTZER AND D. P. GLICK ..... 245
Anthracnose of lupines. J. L. WEIMER ..... 249

Reaction of strains and varieties of barley to many physiologic races of stem rust. F. R. IMMER, J. J. CHRISTENSEN, AND W. Q. LOEGERING ..... 253
Phytopathologic Notes ..... 255
A disease of apple grafts and layers caused by a Rhizoctonia. J. S. COOLEY AND F. B. LINCOLN
Two items of pathological history from California. R. E. SMITH
A new parasite of tomatoes. G. H. STARR

NO. 4. APRIL

Two species of Pythium occurring in southern States. CHARLES DRECHSLER ..... 261
Studies on genotypes of tobacco resistant to the common-mosaic virus. H. H. MCKINNEY ..... 300
Growth of phytopathogenic bacteria in a synthetic asparagin medium. MORTIMER P. STARR AND JAMES E. WEISS ..... 314
A botrytis disease of lupines. J. L. WEIMER ..... 319
Physiologic races of Ustilago nigra. V. F. TAPKE ..... 324
Phytopathological Notes ..... 328
An unusual sporophore of Trametes suaveolens produced on artificially inoculated wood. ELLIS F. DARLEY AND CLYDE M. CHRISTENSEN
A method for maintaining Phytomonas sepedonica in culture for long periods without transfer. ARDEN F. SHERF
On the value of Spergon for seed treatment in small-grain crops. A. W. HENRY
Alternaria sp. on grain kernels killed by high temperature storage. EDGAR BROWN AND ALICE L. ROBERT
Stem rust on Triticum timopheevi. HELEN HART
Book Review ..... 338
War Committee of The American Phytopathological Society—Report of Meeting in Columbus, Ohio, February 13 and 14, 1943 ..... 339

NO. 5. MAY

Blister rust relations of cultivated species of red currants. GLENN GARDNER HAHN 341
Observations on Cercospore leaf spot of tobacco and the question of varietal resistance. RUTH A. MCLEAN ..... 354
Some relationships between potato yellow-dwarf virus and the clover leaf hopper. L. M. BLACK ..... 363
An Alternaria disease of zinnia. A. W. DIMOCK AND JOHN H. OSBORN ..... 372
A description of the fungus causing cotton rust and a preliminary survey of its hosts. JOHN T. PRESLEY AND C. J. KING ..... 382
Diplodia blight in coniferous seedbeds. CHARLES M. SLAGG AND ERNEST WRIGHT ..... 390
Convex gum, a new disease of citrus in China. LIN KUNG-HSIANG ..... 394
The effect of soil and chemical mixtures on the growth of Fusarium oxysporum cubense. CLIFFORD H. MEREDITH ..... 398
The composition of white clover leaves as affected by rust and by sulphur. J. T. SULLIVAN AND S. J. P. CHILTON ..... 401
Phytopathological Notes ..... 403
The antagonism of Actinomyces to Fusarium oxysporum cubense. CLIFFORD H. MEREDITH
Resistance of the genus Nicotiana to Phytophthora parasitica Dastur var. nicotianae Tucker. H. H. FOSTER
Leaf spot on Terminalia arjuna. ANNA E. JENKINS
A differential medium for the isolation of Phytomonas sepedonica. E. A. MARTEN, C. V. LOWTHER AND J. G. LEACH
Leaf blight corn. C. W. ELLETT
Influence of plant populations upon incidence of pineapple yellow spot. M. B. LINFORD
Thiosan (tetramethyl thiuramdisulfide) and scurf control of sweet potatoes. ROBERT H. DAINES
Report of the Annual Meeting of the Council of The American Phytopathological Society for the Year 1942 ..... 413

NO. 6. JUNE

Vapor action of certain fungicidal materials prepared for dusting cotton seed. S. G. LEHMAN ..... 431
A destructive virus disease of sour cherry. G. W. KEITT AND C. N. CLAYTON ..... 449
Pathogenicity studies with Fusaria isolated from tobacco, sweet potato, and cotton. T. E. SMITH AND K. J. SHAW ..... 469

A browning reaction to stem rust in wheat. HELEN HART AND J. LEWIS ALLISON ..... 484
Physical characteristics of Bordeaux mixture in relation to its qualities. E. E. WILSON ..... 497
Tests of eradicant sprays for use against Sclerotinia laxa and Coryneum beijerinckii in apricots and almonds. E. E. WILSON ..... 506
Effects of extract of western red-cedar heartwood on certain wood-decaying fungi in culture. CHESTER M. SOUTHAM AND JOHN EHRLICH ..... 517
Phytopathological Notes ..... 525
The bulb or stem nematode on alfalfa, sweet clover, and white clover. OLIVER F. SMITH AND MERLIN W. ALLEN
Phoma terrestris in the roots of mature maize plants. HELEN JOHANN
Scolescospores in Diplodia macrospora. PAUL E. HOPPE
Plot technique for disease-control studies on fine turf. F. BEN STRUBLE
A method for obtaining single-spore cultures of Agaricus campestris. D. J. DEZEEUW
Host-parasite relationship in rust-infected oats. HARRY B. HUMPHREY AND JEAN DUFRENOY

## NO. 7. JULY

Particle size of sulphur and copper fungicides in relation to apple scab and cedar-apple rust control. J. M. HAMILTON, D. H. PALMITER, AND G. L. MACK ..... 533
Reaction of resistant tobaccos to certain strains of Nicotiana virus 1 and other viruses. H. H. MCKINNEY ..... 551
A study of the genetics of Sorosporium syntherismae and Sphacelotheca panici-miliacei. W. J. MARTIN ..... 569
Severity of curly top in tobacco affected by site of inoculation. W. C. PRICE ..... 586
Comparison of certain mercury and non-metallic dusts for corn seed treatment. P. E. HOPPE ..... 602
Pythium ultimum and the damping-off of cotton seedlings. C. H. ARNDT ..... 607
Phytopathological Notes ..... 612
Freezing preservation of fungi and fungus spores. J. M. HAMILTON AND L. O. WEAVER
"Red ring" of tomato stems caused by an insect, Cyrtopeltis varians (Dist.) at Charleston, S. C. GEORGE B. REYNARD
Southern blight, Corticium rolfsii, of potato tubers. GEORGE F. WEBER
A culture medium of maize-meal and starch mush to replace agar for growing fungi. FREDERICK L. WELLMAN
A reflector scale for measuring growth of fungi. PHILIP HAMM, J. E. MITCHELL, AND DAVID GOTTLIEB
Rasp leaf of cherry. EARLE C. BLODGETT
Book Review ..... 623
Definitions of fungicide terms.
The American Phytopathological Society, Committee on Standardization of Fungicidal Tests ..... 624
The slide-germination method of evaluating protectant fungicides.
The American Phytopathological Society, Committee on Standardization of Fungicidal Tests ..... 627
Standard laboratory Bordeaux mixture.
The American Phytopathological Society, Committee on Standardization of Fungicidal Tests ..... 633

## NO. 8. AUGUST

Evidence for the evolution of phytopathogenic viruses from mitochondria and their derivatives. I. Cytological and genetic evidence. M. W. WOODS AND H. G. DUBUY ..... 637
Root rots in storage of deciduous nursery stock and their control. GEORGE Y. YOUNG 656
Histological studies of infections of the cotton hypocotyl by Glomerella gossypii and Fusarium moniliforme. THOMAS J. HARROLD ..... 666
The sensitivity of plant viruses to certain inactivators. ROBERT W. FULTON ..... 674
Organic materials in pre-harvest sprays for cherries. D. H. PALMITER AND J. M. HAMILTON ..... 683
A tendency to escape tobacco-mosaic disease in derivatives from a hybrid tomato. FRANCIS O. HOLMES ..... 691
A technique to compare virulence of isolates of Alternaria solani on tomato leaflets. FREDERICK L. WELLMAN ..... 698

Ustilago striaeformis. I. Germination of chlamydospores and culture of forma agrostidis on artificial media. K. W. KREITLOW ..... 707
Disappearance of virus from mosaic-diseased sugarcane plants. I. L. FORBES AND P. J. MILLS ..... 713
Mosaic spots of fig fruits. IRA J. CONDIT AND W. T. HORNE ..... 719
Growth substances and the rust fungi. DAVID GOTTLEIB AND HELEN HART ..... 724
A nonchromogenic sporulating variant of Alternaria solani. H. REX THOMAS ..... 729
Chlamydospore germination in the fungus causing dwarf bunt of wheat. C. S. HOLTON ..... 732
Control of Pythium root rot of Aloe variegata by hot-water treatment. KENNETH F. BAKER AND KATHARINE CUMMINGS ..... 736
Phytopathological Notes ..... 739
The production of coacervates. HOWARD S. REED
Breaking in color of flowers of annual phlox caused by the aster-yellows virus. HENRY H. P. SEVERIN
An unusual bean disease. W. J. VIRGIN
A leaf disease of Kentucky bluegrass. ROBERT B. GRIFFITH
Powdery mildew on ribbon-bush (Homalocladium platycladum Bailey). A. W. DIMOCK
Report of the War Committee ..... 747

## NO. 9. SEPTEMBER

A heritable abnormality in the germination of chlamydospores of Ustilago zeae. ST. JOHN P. CHILTON ..... 749
Evidence for the evolution of phytopathogenic viruses from mitochondria and their derivatives. II. Chemical evidence. H. G. DUBUY AND M. M. WOODS ..... 766
Productivity of mosaic-resistant Refugee beans. J. C. WALKER AND J. P. JOLIVETTE ..... 778
The rusts of safflower. I. L. CONNERS ..... 789
Tomato seed treatments in relation to control of Alternaria solani W. D. MOORE, H. REX THOMAS, AND EDWARD K. VAUGHAN ..... 797
Observations on the bacterial crown, stem, and bud rot of delphinium. STEPHEN WILHELM ..... 806
Armillaria root rot of fruit trees in the eastern United States. J. S. COOLEY ..... 812
Influence of contact period on the passage of viruses from cion to stock in Turkish tobacco. C. W. BENNETT ..... 818
Carnation mosaic. D. B. CREAGER ..... 823
Diplodia pinea and Sphaeropsis malorum on soft pines. ALMA M. WATERMAN ..... 828
Sphaerotheca humuli var. fuliginea on delphinium in California. KENNETH F. BAKER ..... 832
Phytopathological Note ..... 835
Mercury compounds applied to banana plants in the field. CLIFFORD H. MEREDITH

## NO. 10. OCTOBER

Comparative symptomatology of psorosis varieties on citrus in California. H. S. FAWCETT AND A. A. BITANCOURT ..... 837
The relations of wood alterations in psorosis of citrus to tree deterioration. A. A. BITANCOURT, H. S. FAWCETT AND J. M. WALLACE ..... 865
Population trends of physiologic races of Puccinia graminis tritici in the United States for the period 1930 to 1941. E. C. STAKMAN, W. Q. LOEGERING, R. C. CASSELL, AND LEE HINES ..... 884
A comparison of laboratory and field retention and protective value of certain copper fungicides. HAROLD J. MILLER ..... 899
Studies of the susceptibility of forage grasses to cereal smut fungi. IV. Cross-inoculation experiments with Urocystis tritici, U. occulta, and U. agropyri. GEORGE W. FISCHER AND C. S. HOLTON ..... 910
Estimation of the leaf area of potato plants for pathological studies. J. G. BALD 922
Boron deficiency in the olive. C. EMLEN SCOTT, H. EARL THOMAS, AND HAROLD E. THOMAS ..... 933
The inheritance of a white mutant character in Ustilago zeae. E. C. STAKMAN, M. F. KERNKAMP, W. J. MARTIN, AND T. H. KING ..... 943
The nature of ultra-viruses and their biological activity. V. L. RISCHKOV ..... 950
A new species of Fusarium causing vascular wilt of tomato. FREDERICK L. WELLMAN ..... 956
A bacterial leaf spot and blight of the Russian dandelion. JOHN S. NIEDERHAUSER ..... 959

Phytopathological Notes ..... 962
Prevention of three peach diseases by ferric dimethyldithiocarbamate spray. E. E. WILSON AND C. E. SCOTT
Bacterial infection and decay of the inner wood of winter-injured young London plane trees. BOWEN S. CRANDALL

## NO. 11. NOVEMBER

Decay in merchantable black cherry on the Allegheny National Forest. Ross W. DAVIDSON AND W. A. CAMPBELL ..... 965
The fungus causing the so-called "Septoria leaf-spot disease" of raspberry. J. B. DEMAREE AND MARGUERITE S. WILCOX ..... 986
Comparative toxic effects of extracts from mild and virulent isolates of tomato-wilt Fusarium. FREDERICK L. WELLMAN ..... 1004
Diplodia pinea, the cause of a disease of hard pines. ALMA M. WATERMAN ..... 1018
Studies on the morphology, physiology, serology, longevity, and pathogenicity of Corynebacterium sepedonicum. S. F. SNIESZKO AND REINER BONDE ..... 1032
Acute and chronic symptoms in tobacco mosaics. H. H. MCKINNEY AND E. E. CLAYTON ..... 1045
Ustilago striaeformis, II. Temperature as a factor influencing development of smutted plants of Poa pratensis L. and germination of fresh chlamydospores. K. W. KREITLOW ..... 1055
New and standard seed treatments in the control of certain seedborne diseases of wheat, oats, and barley. F. J. GREANEY AND H. A. H. WALLACE ..... 1064
A new method for computing sugar beet leaf area. WILLIAM DOWELL BATEN AND J. H. MUNICE ..... 1071
Distribution of bacterial wilt (Bacterium solanacearum) in successive crops of tobacco grown on the same fields. T. E. SMITH ..... 1076
Rhizoctonia root canker of alfalfa (Medicago sativa). OLIVER F. SMITH ..... 1081
An Andean disease of potato tubers. M. F. BARRUS AND A. S. MULLER ..... 1086
Some miscellaneous diseases of mushrooms. A. M. KLIGMAN AND J. S. PENNY ..... 1090
A water-soluble protectant fungicide with tenacity. ALBERT E. DIMOND, JOHN W. HEUBERGER, AND JAMES G. HORSFALL ..... 1095
Control of soil-borne organisms that cause rots of garden peas. M. F. BABB AND G. W. BOHN ..... 1098
A noninfectious heritable leaf-spot and shot-hole disease of the Beaty plum. C. O. SMITH AND L. C. COCHRAN ..... 1101
Severe potato late-blight infection in Sebago tubers. G. H. RIEMAN AND JOHN S. MCFARLANE ..... 1104
Announcement ..... 1107

## NO. 12. DECEMBER

Abstracts of papers presented at the thirty-fifth annual meeting of the society, Columbus, Ohio, December 4–6, 1943 ..... 1109
Studies on cytology of Ustilago crameri. C. S. WANG ..... 1122
Some factors inhibiting the fructification and production of the cultivated mushroom, Agaricus campestris L. E. O. MADER ..... 1134
The function of lime and host leaves in the action of Bordeaux mixture. C. E. YARWOOD ..... 1146
Typhula snowmold of pasture grasses. C. C. WERNHAM AND ST. JOHN P. CHILTON ..... 1157
Soil fumigation with chloropicrin for control of the root knot nematode, Heterodera marioni. A. L. TAYLOR ..... 1166
Some cultural practices that influence the development of Alternaria solani on tomato seedlings. W. D. MOORE AND H. REX THOMAS ..... 1176
Effect of mineral nutrition on flower production of own-rooted roses and the incidence of black-spot. E. O. MADER ..... 1185
Eradicant action of fungicides on spores on living plants. E. M. STODDARD AND J. W. HEUBERGER ..... 1190
A graft-transmissible mosaic of American elm. R. U. SWINGLE, P. E. TILFORD, AND C. F. IRISH ..... 1196
A hitherto unreported disease of the Washington elm. LEO PINE ..... 1200
Inhibition of Phymatotrichum sclerotia formation by sulphur autoclaved with soil. A. A. DUNLAP ..... 1205
Association between Nematospora phaseoli and the green stinkbug. J. G. LEACH AND GENEVIEVE CLULO ..... 1209

Phytopathological Notes ..... 1212
Occurrence of the imperfect stage of Sclerotinia laxa on Prunus cerasus in Wisconsin. G. W. KEITT, J. DOUAIN MOORE, E. C. CALAVAN, AND J. R. SHAY
Masking of leaf symptoms of sour-cherry yellows by temperature effects. G. W. KEITT AND J. DUAIN MOORE
A new mosaic disease of beans. B. LORIN RICHARDS AND WALTER H. BURKHOLDER
A foliar mottle and necrosis in Chippewa potatoes associated with infection by a strain of the potato X virus. R. H. LARSON

# INDEX FOR VOLUME 33

New species in **blackface type**
Joint authorship indicated by pages in "( )"
Species index of bacteria included
AUTHOR AND SUBJECT INDEX

Prepared by Frederick V. Rand, Office of Experiment Stations, U. S. Department of Agriculture


Acer rubrum, decayed wood, calorific and chemical study, 45
Aceratogallia sanguinolenta, as vector of: potato yellow dwarf, 17, 363
potato yellow dwarf vars., 1110
Actinomyces sp., lysis of Fusarium oxysporum cubense by, 403
Aecidium carthamni, on safflower, 795
Aeration, root-, cantaloupe physiological leaf spot role, 162
Aerobiology, spore identification from exposed slides, 75
Agallia constricta, as vector of: potato yellow dwarf, 17
potato yellow dwarf vars., 1110
Agar, substitute, corn-meal and starch mush, 617
Agaricus campestris, disease resistance, mechanism, 13
fructification and mushroom production, inhibitory factors, 1129
single-spore culture method, 530
spore germination method, 530
Age, plant-, effect on incidence of tomato early blight, 1176
Agrobacterium spp., growth in synthetic medium as taxonomic aid, 315
Agropyron spp., smuts of cereals on, reactions to, 912
cristatum, Helminthosporium sativum on, 238
Pythium graminicolum on, 238
Agrostis alba, stripe smut, chlamydospore germination, 707
culture, 7, 707
Air, spores on slides exposed to, identification, 75
Air dissemination of: carrot Cercospora blight, 118
Hypoxylon pruinatum, 1112
pineapple yellow spot vector, 410
wheat stem rust, 12
Alfalfa, Ditylenchus dipsaci strain on white clover, sweet clover and, 525
Rhizoctonia root canker, general study, 1081
Alkyl mercuric acetylene, fungicidal action of vapor, 445
Alkyl mercuric acetylene urea, fungicidal action of vapor, 445
ALLEN, MERLIN W., (525)
ALLEN, T. C., 1109
ALLISON, J. LEWIS, (484)
Almond, Coryneum beijerinckii on, eradicant sprays for, 506
Sclerotinia laxa on, eradicant sprays for, 506
Aloe variegata, Pythium root rot control by hot water, 736
Alternaria spp., on cereal seeds, storage temp. effects, 333
in deciduous nursery tree root rot and control, 656
wheat kernel smudge due to, varietal reactions, 4
solani, on Lycopersicon spp., "physiological" maturity vs., 1
on potato, zinc sulphate-lime effect on fungicidal control, 1113
on tomato, control, by Fermate, 1119
by seed treatments, 797
culture practices influencing, 1176
sporulating non-chromogenic variant, 729
virulence of strains, comparison method, 698
zinniae, distribution, 372
seed and soil transmission, 379
on Zinnia elegans, general study, 372
Althaea rosea, Puccinia stakmanii (exp.) on, 387
AMERICAN PHYTOPATHOLOGICAL SOCIETY, annual report (1942), 413
War Committee reports, 339, 747
Ammonium compounds, cationic quarternary, fungicidal value, 1115
Anaerobiosis, sap collection under, technique, 129
Anatomy, plant-, book review, 623
ANDERSON, H. W., 1109
ANDREWS, E. A., 234
angulatum (Bacterium), 1120
Annulus tabaci, cyanide effects on synthesis, 77
Antagonisms, Actinomyces for Fusarium, 403
clavacin for phytopathogenic bacteria, 1109
crop-residue toxicity, microbial role, 1111
in Pythium spp., 287
between toxicants, significance, 18
viruses vs. "non-toxic" substances, 675
Anthracnose, cotton-, 666
honeydew melon-, 245
lupine-, 249
Rubus, 1115
tobacco-, in plant beds, 15
tomato-, 1120
Antibiosis (see Antagonisms)
Antiseptic, 8-hydroxyquinoline as, mechanism, 1121
Aphanomyces sp., damping-off by, measure of, seedling germination rate/fungus growth rate as, 7

cochlioides, sugar beet seedling disease due to (?), 7
Apple, Armillaria root rot in eastern U. S. A., 814
cold-storage behavior, boron effects, 2
fungicides on, field testing technique, 13
redistribution on leaves, significance, 5
growth and yield, copper fungicide effects, 56
sulphur fungicide effects, 2, 56
internal bark necrosis, boron vs., 1
Rhizoctonia on grafts and layers, 255
rust, control, by Fermate, 5
particle size vs., 533
on Wealthy var. in Minn., 1118
scab, control, by eradicant vs. protectant sprays, 6
eradicant action of fungicides on leaves for, 1192
by eradicant spray, cationic phenyl Hg compounds as, 1114
by Fermate, 5
fungicide particle size vs., 533
sulphur coverage effect vs., 1119
water-soluble fungicide for, 1097
genetics of fungus, 11
incidence on Wealthy var. in Minn., 1118
inheritance in fungus, 19
spraying, efficiency of new-type equipment, 1114
Apricot, Coryneum beijerinckii on, eradicant sprays for, 506
Sclerotinia laxa on, eradicant sprays for, 506
Area determination, on leaves, technique, 922, 1071
Armillaria sp., fruit tree root rot due to, in eastern U. S. A., 812
ARNDT, C. H. 607
ARRUDA, S. C., 1
Arthur, Joseph Charles, biographical note, 428
Ascorbic acid, effect on rust fungi, 724
Aspen, Hypoxylon pruinatum on, pathogenicity factors, 1112
Aspergillus niger, growth product, effect on plant viruses, 675
Aster, yellows, on Ambrosia artemisiifolia, 16
virus, annual-phlox infection via leaf hoppers, 741
potato infection, 16
properties, 2
transmission by Macrosteles divisus, 16

BABB, M. F., 1098
Bacteria, in culture, maintenance technique, 330
identification by bacteriophage, 1119
phytopathogenic, clavacin effects on, 1119
growth in synthetic medium as taxonomic aid, 314
"Bacterial pit," of mushrooms, description and etiology, 1092
Bactericides, clavacin, effect on phytopathogens, 1119
Bacteriophage, bacteria identified by, 1119
Bacteriostatic action, of 8-hydroxyquinoline, mechanism, 1121
Bacterium angulatum, on roots of pasture plants, 1120
solanacearum, in tobacco, control by urea in soil, 11
pattern of occurrence in successive plantings, 1076
tabacum, multiplication on roots other than tobacco, 3
overwintering in soil, 1109
pathogenicity to tobacco and serology of miscellaneous bacteria vs., 1109
on roots of pasture plants, 1120
tumefaciens, invading tumors induced by, factors influencing, 8
BAIN, D. C., 220
BAKER, KENNETH F., 736, 832
BALD, J. G., 922
BALDWIN, HENRY IVES, 338
Banana, soil applications of mercurials, Fusarium oxysporum cubense vs., 835
Banded corky bark, citrus-, virus (?) origin, 862
Barbak, D., corn seed treatment by, 602
Bark disorders, citrus-, virus (?) origin, 860
Barley, Alternaria on seeds, storage temp. effects, 333
Helminthosporium sativum on seed in Canada, 4
loose smuts, occurrence, identification and species validity, 194
powdery mildew, on grass spp., 5
seed-borne diseases, seed treatments for, 1064
stem rust, adult vs. seedling reactions, 254
reactions to races, 253
Ustilago nigra on, physiologic races, frequency and distribution, 324
BARRATT, R. W., 1
BARRUS, M. F., 1086
BARSS, HOWARD P., 420
BATEN, WILLIAM DOWELL, 1071
BEACH, W. S., 1109
BEALE, HELEN PURDY, (8)
Bean, Lima, downy mildew, fungicides for, 2
seed treatment vs. soil moisture, 3
yeast spot Nematospora, association with green stinkbug, 1209
Macrophomina dry rot, 1113
mosaic virus, sensitivity to inactivators, 675
virus-4, symptom expression, genetics of, 16
mosaic-resistant, productivity and quality of snap vars., 778
pea-, mosaic (new) of, 1215
powdery mildew, fungicidal action on, factors in, 1148
rust, fungicidal action on, factors in, 1147
variegation resembling mosaic in snap-, 779
virus (?) disease (new), 743
wax-, blossom drop reduced by growth substances, 1109

Bean virus-2, new bean virus (?) related to, 743
Beauveria bassiana, on elm insects, distribution in U.S.A., 76
Beech, decayed wood, calorific and chemical study, 45
Beet (see Sugar beet)
BENNETT, C. W., 424, 818
Benzine, fungicidal action of vapor, 445
Benzol, fungicidal action of vapor, 445
BERG, ANTHONY, 1
Beta-indoleacetic acid, effect on rust fungi, 724
Betula papyrifera, decayed wood, calorific and chemical study, 45
Bignonia capriolata, Sphaceloma on, 1
Biographies and necrologies, Arthur, Joseph Charles, 428
Mitra, Manoranjan, 429
Pierstorff, Arthur Lewis, 430
BIOLOGICAL ABSTRACTS, committee report on, 419
Birch, white- (see Betula papyrifera)
BITANCOURT, A. A., 1, (837), 865, 1109
BLACK, L. M., 2, 17, 363, 1110
"Black point," wheat-, 1112
Black shank, tobacco Phytophthora, 403
Black spot, rose-, 1097
Blackberry, Haplosphaeria stamen blight, distribution, symptoms, pathogenesis, and control, 136
Septoria rubi leaf spot, 1002
Blackfire, tobacco-, 1120
Black-spot, rose-, 1185
Blights, arborvitae (oriental), Cercospora causing, 1117
barley Helminthosporium seedling-, 1064
blackberry Haplosphaeria, 136
carrot Cercospora, 114
celery Cercospora, 1097
chestnut-, 18
corn (field) Helminthosporium leaf-, 18
dewberry Haplosphaeria, 136
eggplant Phomopsis, 1117
grass fungus seedling-, 234
peach Coryneum, 962
peach Sclerotinia blossom-, 15
pine Diplodia, 390
potato early-, 1113
potato late-, 1104
potato southern (Corticium), 615
stone-fruit Coryneum, 1120
stone fruit Sclerotinia, 506
tomato Alternaria, 1, 698, 729, 797, 1119, 1176
tomato leaf-, 9
wheat Helminthosporium seedling-, 1064
Blind-pocket psorosis, comparative symptomatology, 854
BLODGETT, EARLE C., 620
Blossom drop, in wax bean, growth substances reducing, 1109
Blotch, mushroom bacterial, 1093
Bluegrass, Kentucky- (see Poa pratensis)
Ustilago striaeformis on, isolation and culture, 1116
Blue mold, tobacco- (see Peronospora tabacina)
BOHN, G. W., (1098)
BONDE, REINER, (1032), 1110, 1118
Book reviews (see under Reviews)
Bordeaux mixture (see under Fungicides)
Boron, apple internal bark necrosis vs., 1
effect on flax diseases, 1113
Boron deficiency, apple-, effect on apple cold-storage behavior, 2
olive-, general study, 933
rutabaga water-core due to, control, 8
Botrytis cinerea, on cherry, organic materials for pre-harvest spray, 683
on lupines, 319
Bouteloua spp., Puccinia stakmanii (exp.) on, 387
gracilis, Helminthosporium sativum on, 238
BRAUN, ALVIN J., (136), (156)
BRAUN, ARMIN C., 85
Breakdown, sugar cane internal-, mosaic-associated, 3
"Breaking," in phlox, aster yellows virus via leaf hoppers causing, 741
Breeding (see also Genetics, Mutation)
chestnut, for blight resistance, 18
tobacco, for mosaic resistance, 567
tomato, for tobacco mosaic resistance, 691
BREMER, H., 165
BRENER, W. H., (11)
BRISCHLE, H. A., (4)
Bromus inermis, seedling blight and root rot, 238
Broom-rape, tomato parasitized by, 257
BROWN, EDGAR, 333
Browning reaction, in wheat stem rust, 484
"Buba," potato disease of Andean region, 1086
Bunt, wheat-, 240, 259, 332
wheat dwarf-, 732
BURKHOLDER, WALTER H., (1215)
BURRELL, A. B., 2
BYAM, L., (11)

Cabbage, yellows, host nutrition role, 15
CALAVAN, E. C., (1212)
Camellia spp., Sphaceloma on, 1
CAMPBELL, W. A., (965)
Canker, lupine Botrytis, 319
pine (soft) Diplodia, 828
Cantaloupe, physiological leaf spot, root aeration role, 162
seed protectants for, 14
Carbon disulphide, soil adsorption, 12
soil sterilization by, wetting agent effects on, assay, 11
Carduncellus caeruleus, Puccinia carduncelli on, 792
Carnation, mosaic, symptoms, virus nature, transmission and distribution, 823
rust, eradicant action of fungicides on leaves for, 1191
carotovora (Erwinia), 80, 1120
Carrot, Cercospora blight, dissemination, pathogenesis, hosts, and general study, 114
mineral nutrition relations, 121
seed treatment for, 119
seed protectants for, 14

Carthamnus calvus, Puccinia carduncelli on, 792
tinctorius, rusts of, 789
CARVAJAL, F., 2
CASSELL, R. C., (884)
Catalpa bignoniodes, Sphaceloma on, 1
Catex, onion smut control by, 9
Cattelya spp., Erwinia soft rot, 80
Cattle, serum of, effect on plant viruses, 675
Cedar, western red- (see Thuja plicata)
Cedar-apple rust, fungicidal control, particle size vs., 533
Celery, Cercospora blight, water-soluble fungicide for, 1097
Cells, coacervates in plant-, origin and artificial production, 739
Cenchrus pauciflorus, smut, cross inoculations, 572
Ceratostomella ulmi, on elm, internal therapy, 16
spore dosage and elm response, logarithmic-probit relations, 1121
toxin of, virus compared, 20
Cercospora spp., arborvitae (oriental) blight due to, 1117
cypress (Italian) blight due to, 1117
apii, water-soluble fungicide for, 1097
carotae, dissemination, pathogenesis, host and physiology, 114
nicotianae, on tobacco, enviromal relations, 355
resistance to, real vs. apparent, 358
oryzae, physiological races, 70
Cereals, Alternaria on seeds, storage temp. effects, 333
seed treatments, report of coordination-of-research committee, 423
by Spergon, 332
seed-borne diseases in Manitoba, 1111
smuts, grass susceptibility to, 910
Typhula snowmold, general study, 1157
Ceresan, fungicidal action of vapor, 431
Chalara sp., oaks rapidly killed by, 18
Chemotherapy, internal, for vascular diseases, 16
CHEN, SHAN-MING, 1118
Cherry, Armillaria root rot in easter U. S. A., 814
black- (see Prunus serotina)
fungicides on, field testing technique, 13
leaf spot, spray program vs. epidemiology, control, injury and fruit size, 1115
Montmorency, leaf spot control as influenced by copper spray dosage and injury, 9
pre-harvest spray, organic materials in, 683
rasp leaf, virus nature, distribution and description, 620
sour-, Sclerotinia laxa imperfect stage on, in Wis., 1212
yellows, now virus disease, 449
symptom masking by temp, 1213
virus diseases, 6
yellows, insect transmission, 466
strains in Montmorency var., 6
Cherry-plum hybrids, Sclerotinia fructicola from, variation in single-ancospore isolates, 1118
CHESTER, K. STARR, 17, (1113)
Chestnut, blight resistance in timber type, breeding for, 18
CHILDS, W. H., 1119
CHILTON, ST. JOHN P., (171), (401), 749, (1157)
Chlamydospores, abnormality inherited in Ustilago, 749
Chlorophenates, as eradicant sprays for stone fruit fungi, 1120
Chloropicrin, fungicidal action of vapor, 445
root knot control by, in greenhouse, 6
soil fumigation, 1166
soil adsorption, 12
soil sterilization by, wetting agent effects, assay, 12
Chloroplasts, isolation method, 766
Chondriome, phytopathogenic viruses evolved from (?), 20
CHRISTENSEN, CLYDE M., (328)
CHRISTENSEN, J. J., (253), (1112), (1117)
Chromoprotein, extraction from plastids, 768
properties, 769
Chrysanthemum, Verticillium-free clones, establishment, 3
Circular-spot corky bark, citrus-, virus (?) origin, 862
Citrivir psorosis var. alveatum, comparative symptomatology, 854
var. anulatum, comparative symptomatology, 847
var. concavum, comparative symptomatology, 850
var. vulgare, comparative symptomatology, 840
Citrus, bark disorders due to viruses (?), 860
convex gum (virus?) of, 394
infectious variegation, crinkle leaf association, 860
psorosis, comparative symptomatology of forms of, 837
wood alteration vs. tree deterioration, 865
Clavacin, effect on phytopathogenic bacteria, 1109
Claviceps sp., on Zizania, 12
purpurea, epidemic production technique, 1116
CLAYTON, C. N., (449)
CLAYTON, E. E., (8), (11), 101, (1045)
Clones, Verticillium-free establishment, 3
Clover, white- (see Trifolium repens)
CLULO, GENEVIEVE, (1), (1209)
Coacervates, in plant cells, origin and artificial production, 739
COCHRAN, L. C., (1101)
Colletotrichum sp., in tobacco anthracnose, 15
falcatum, Physalospora perfect stage, 2
lagenarium, on honeydew melons as influenced by packing methods, 245
phomoides, on tomato, effect on leaf blight spray control, 9

COLLEY, REGINALD H., 17
Compost, effect on Fusarium-root knot-attacked cotton yields, 10
Concave-gum psorosis, citrus-, comparative symptomatology, 850
CONDIT, IRA J., 719
Coniophora cerebella, black cherry rot due to, general study, 981
puteana, as influenced by western red cedar heartwood extracts, 518
CONNERS, I. L., 789
Convex gum, citrus virus (?) disease, 394
COOLEY, J. S., 255, 812
Copper, fungicidal (see under Fungicides)
Cordwood, decayed, calorific and chemical study, 45
Corky bark, citrus-, virus (?) origin, 860
Corn, Diplodia macrospora on, in Md., 528
field-, Helminthosporium turcicum leaf blight of inbreds and hybrids, 18, 407
Phytomonas stewartii leaf blight of imbreds and hybrids, 407
inoculation method for ear and stalk rots, 16
Macrophomina dry rot, 1113
pink roots on, Phoma terrestris causing, 526
smut, chlamydospore abnormality, 749
white mutant, 943
seed treatment by mercury vs. non-metallic dusts, 602
sweet-, seed protectants for, 14
Corticium rolfsii, potato southern blight due to, description and soil moisture relations, 615
solani, sugar beet leaf blight due to, 6
Corynebacterium spp., phytopathogenic, growth in synthetic medium as taxonomic acid, 315
sepedonicum, culture in potato-juice media, 1118
morphology, physiology, serology, longevity, and pathogenicity, 1032
survival in storage, 1110
taxonomy, 1042
Coryneum beijerinckii, chlorophenates as eradicant sprays for, 1120
on peach, control by Fermate, 962
on stone fruits, eradicant sprays for, 506
COTTER, R. U., (12)
Cotton, damping-off, by Pythium ultimum, conditions favoring, 607
seed treatment for, 51
Fusarium-root knot on, soil amendment effects on yield and, 10
Fusarium wilt, crop rotations for, 483
distribution, 469
on sweet potato and tobacco vs., 469
hypocotyl infection by Glomerella gossypii vs. Fusarium moniliforme, histopathology, 666
lightning injury, Rhizoctonia associated, 154
types, symptoms, and effects, 150
Rhizoctonia infection, seed dusting against, 51
rust (new), distribution, hosts and general study, 382
seed treatments, 17
by fungicidal vapors, 431
Cover crops, tobacco leaf-spot bacteria on roots of, 1120
Cowpea, Macrophomina dry rot, 1113
Cranberry, false blossom, transmission from tomato by dodder, 19
CRANDALL, BOWEN S., 963
CREAGER, D. B., 823
Creosote dust, fungicidal action of vapor, 445
Crinkly-leaf psorosis, comparative symptomatology, 858
Cronartium ribicola, on red currant spp. and vars., relationships, 341
Ribes distribution measurement in control work, 4
on white pine, red currant role, 341
selection for resistance, 11
Crop residues, effects on root diseases, 1111
Crown gall, chemical influences resembling, 1119
invading forms and factors influencing, 8
malignancy in animal tumors vs., 98
sterility of secondary tumors, 85
Crumbly-gum corky bark, citrus-, virus (?) origin, 860
Cucumber, mosaic, virus, passage from cion to stock, contact period effects, 818
sensitivity to inactivators, 675
powdery mildew, fungicidal action on, factors in, 1147
tobacco ring spot on, acute vs. chronic symptoms, 8
Cucumis virus-1, reactions of resistant tobaccos to, 565
Cultures, media (see under Technique)
technique (see under Technique)
CUMMINGS, KATHERINE, (736)
CUNNINGHAM, H. S., 2, 3
Cupressus sempervirens, Cercospora blight, 1117
Curly top, on tobacco, immunity acquirement mechanism, 589
inoculation site effects, 10, 586
virus, passage from cion to stock, contact period effects, 818
Currants, red-, blister rust relations of cultivated spp., and vars., 341
CURTIS, LAWRENCE C., 1110
Cuscuta campestris, cranberry false blossom transmitted by, 19
Cyanamid, effect on Fusarium-root knot-attacked cotton yields, 10
Cyanide, effect on tobacco virus synthesis, 77
Cylindrosporium rubi, conidial stage of Sphaerulina rubi, 997
Cypress, Italian (see Cupressus sempervirens)
Cyrtopeltis varians, distribution, 614
tomato stem "red ring" due to, 613

DAINES, ROBERT H., 410
Daldinia sp., cordwood decay by, calorific and chemical study, 46
Damping-off, cotton Pythium, 607
cotton Rhizoctonia, 51
Macrophomina-induced, 1113
by oomycetes, antibiotic relations among pathogens, 261
of pine in nursery, 11
post-emergence-, vegetable seed protectants for, 14
preemergence-, measure of, seedling germination rate/fungus growth rate as, 7
sodium nitrite soil treatments for, 1110
Dandelion, Russian, bacterial leaf spot (new), 959
DARLEY, ELLIS F., 328
Daucus spp., Cercospora carotae on, general study, 114
DAVIDSON, ROSS W., 965
DAVIS, WILLIAM C., 338
Deciduous tree nursery stock, root rot and control, 656
Decline disease, raspberry virus-, symptoms, transmission and control, 156
Deficiency diseases, boron, of apple, 2
in California crops, 939
of olive, 933
in rutabaga, control, 8
Delphiniums, bacterial crown, stem and bud rots, factors influencing, 806
varietal reactions, 810
Sphaerotheca humuli var. fuliginea on, distribution and rapid identification, 832
DEMAREE, J. B., 986
Dewberry, Haplosphaeria stamen blight, distribution, symptoms, pathogenesis and control, 136
Septoria rubi leaf spot, 1002
DE ZEEUW, D. J., 530
DIACHUN, STEPHEN, 3, (1120)
Dicotyledons, mycorrhizas of, in Colorado, 146
Die-back, pine Diplodia, 1018
DIMOCK, A. W., 3, 372, 745
DIMOND, ALBERT E., 18, 1095
Diplocarpon rosae, on rose, mineral nutrition effects, 1185
water-soluble fungicide for, 1097
Diplodia macrospora, with scolecospores, on corn in Md., 528
pinea, on hard pines, taxonomy, hosts, distribution and pathology, 1018
pine nursery and mature tree blight due to, 390
on soft pines, significance, 828
zeae, on corn, inoculation method, 16
seed treatment by mercury vs. nonmetallic dusts, 602
Diseases (plant), bacterial identification by bacteriophage, 1019
coacervates in, origin and artificial production, 739
deficiency (see Deficiency diseases)
escape, heritable, in tobacco mosaic on Lycopersicum chilense and hybrids, 19, 691
grass-, on fine turf, control studies, plat technique, 528
incidence, age effects in tomato early blight, 1177
in living wood, detection by dyes, 20
mushroom-, resistance to, mechanism, 13
pea-, survey in Wis., 15
physiological (see Physiological diseases)
regulatory work and foreign, committee report, 422
seed-borne, of forest trees, book review, 338
symptoms in virus-, acute vs. chronic, 8
vascular, therapy, internal chemical, 16
Disodium ethylene bisdithiocarbamate, as water-soluble protectant fungicide, 1095
Distribution of: Alternaria zinniae, 372
Armillaria fruit tree root rot, 812
Beauveria bassiana on elm insects in U. S. A., 76
carnation mosaic, 823
cereal seed-borne diseases, 1011
Chalara sp. on oaks, 18
cherry rasp leaf, 622
Cyrtopeltis varians, 614
Diplodia macrospora, 528
Diplodia pinea, 1020
Fusarium wilts of cotton, sweet potato and tobacco, 469
Gloeocercospora sorghi, 222
Haplosphaeria deformans, 136
Helminthosporium sativum, 4
Peronospora tabacina, 108
Puccinia carthami, 789, 792
Pythium myriotylum, 261
Ribes spp., 4
Sclerotinia laxa, 1213
Septoria oudemansii, 745
Sphaceloma violae, 168
Sphaerotheca humuli var. fuliginea, 832
Titaeospora andropogonis, 220
Uromyces betae, 10
Ustilago spp. on barley, 196, 205, 206
Ustilago nigra, physiological races, 326
Ditylenchus dipsaci, on onion, pathogenesis, 61
strain on alfalfa, white clover, and sweet clover, 525
Dodder, cranberry false blossom transmitted by, 19
DRECHSLER, CHARLES, 227, 261
Dry rot, potato Fusarium, charcoal rot association, 1120
DUBUY, H. G., (20), (637), 766
DUFRENOY, JEAN, (3), (531)
DUNLAP, A. A., 1205
Dutch elm disease (see Ceratostomella ulmi)
Dyes, abnormal tissues in living wood detected by, 20

EDGERTON, C. W., (1), (2), (220)
Eggplant, Phomopsis blight, Fermate-talc dust control, 1117
Verticillium wilt, internal therapy, 16
EHRLICH, JOHN, (517)
Elgetol, as eradicant for pear sooty blotch, 1117
ELLETT, C. W., 407
ELLIOTT, CHARLOTTE, 18
ELLIS, D. E., 1110

Elm, Dutch elm disease, internal therapy, 16
spore dosage and response in, logarithmic-probit relations, 1121
toxin of, virus compared, 20
insects of, Beauveria bassiana distribution in U. S. A. on, 76
mosaic, graft-transmissible, 1196
Elsinoë **n. sp.**, mango scab due to, 1
veneta, perfect stage of Gloeosporium necator, 1115
Elymus spp., smuts of cereals on, reactions to, 912
Endothia parasitica, on chestnut, breeding for resistance, 18
Ergot, epidemics of, technique of producing, 1116
on Zizania, 12
Erwinia carotovora, orchid soft rot due to, 80
potato charcoal rot association, 1120
phytophthora, delphinium crown, stem, and bud rots due to, factors influencing, 806
Erysiphe graminis, races, pathogenicity on Hordeae, 5
polygoni, fungicidal action on, factors in, 1148
ribbon-bush powdery mildew due to (?), 745
Etch, tobacco, hosts (exp.), 1114
Ethide, soil sterilization by, wetting agent effects on, assay, 11
Ethyl ether, fungicidal action of vapor, 445
Ethylene dichloride, soil sterilization by, wetting agent effects on, assay, 12
Evolution, of viruses, 20, 637, 767
EXNER, BEATRICE, 3, 171
Experimental technique (see under Technique)

False blossom, cranberry-, transmission from tomato by dodder, 19
FAWCETT, H. S., 837, (865)
FELDMAN, A. W., 1111
Fermate, apple frog-eye leaf spot control by, 1116
apple rust control by, 5
apple scab control by, 5
as cherry pre-harvest spray, 683
grape downy mildew control by, 13
peach disease control by, 962
seed-protectant value, 9
talc dust for eggplant blight, 1117
tomato anthracnose control by, 1120
tomato early blight control by, 1119
as vegetable seed protectant, 14
Fermate-Pyrax AAB mixture, tomato early blight control by, 1119
Ferric-dimethyl-dithio-carbamate (see Fermate)
Fig, mosaic spot, 719
FISCHER, GEORGE W., 910
FISHER, ELLSWORTH, (1109)
Flax, diseases, boron effects on, 1113
seed treatment, seed injury vs., 1117
by Spergon, 332
Flower production, mineral nutrition effects on rose-, 1185
Fluids, plant-, anaerobic collection method, 129
fluorescens (Pseudomonas), 1091
Fomes spp., as influenced by western red cedar heartwood extracts, 518
pinicola, black cherry rot due to, general study, 981
Foot rot, wheat Helminthosporium, 4
FORBES, I. L., 3, 713
Forestry, tree seeds, book review, 338
Formaldehyde, soil adsorption, 12
soil sterilization by, wetting-agent effects on, assay, 12
FOSTER, ADRIANCE S., 623
FOSTER, H. H., 403
FRACKER, S. B., 4
Freezing, viability of fungi and spores preserved by, 612
FRIEDMAN, BERNARD A., (80)
Fruit trees (see under Trees)
FULTON, ROBERT W., 674
Fumigants, for mushroom truffle disease, 13
soil-, for root knot, 1118, 1166
wetting-agent effects on, assay, 12
soil adsorption, 12
Fungi (see also Mycology)
on air-exposed slides, identification, 75
antibiotic relations in oomycetes, 287
on cereal seeds, heat treatment for, 333
in culture, maintenance technique, 330
growth, reflector scale for measuring, 619
as influenced by vitamins and growth substances, 724
inheritance in (see under Genetics)
media for, agar substitute, 617
spores, eradicant action of fungicides for, on living leaves, 1190
toxins of viruses vs., 20
viability of spores and, preservation by quick freezing and storage, 612
wood-rotting, calorific and chemical effects, 45
as influenced by heartwood extracts of western red cedar, 517
preservatives against, 17
Fungicides (see also Pesticides)
assay, of Bordeaux, 633
correlation of greenhouse and laboratory methods, 15
of effectiveness, 5
factors influencing, 1154
laboratory-greenhouse method, 19
slide-germination method for protectant, 627
Catex, for onion smut control, 9
cationic quaternary $NH_4$ compounds as, 1115
comparisons of lime, $CuSO_4$ and Bordeaux, 1146
copper-, Bordeaux, action as influenced by lime and host leaves, 1146
guttation-fluid effects on, 1110
physical characters vs. quality, 497
standard laboratory mixture, preparation and use, 633
weathering vs. preparation method, 503
early use in U. S. A., 258
effect on apple growth and yield, 56

for grapes, disease control vs. spray injury, 13
particle size, infection control vs., 533
protective value and retention, field and lab. comparisons, 899
status of fixed-, 1121
efficiency, coverage factor, determination technique, 1114
eradicant, action on fungus spores on living leaves, 1190
chlorophenates as, on stone fruits, 1120
for Coryneum beijerinckii, 506
Elgetol as, for pear sooty blotch, 1117
mechanism of action, determination techniques, 1194
for Sclerotinia laxa, 506
Fermate (see Fermate)
ferric - dimethyl - dithio - carbamate, (see Fermate)
field testing on fruit trees, technique, 13
juglone, evaluation, 1112
on leaves, redistribution significance, 5
mercurial, cationic phenyl compounds as apple scab eradicants, 1114
corn seed treatment by, non-metallic dusts compared, 602
soil-application effects on banana vs. Fusarium oxysporum cubense, 835
vapor action, 431
mercurial organic, seed treatment for cereal diseases by, 1064
new-type spray equipment for, efficiency, 1114
organic, for downy mildew of lima bean, 2
in pre-harvest cherry sprays, 683
sweet potato scurf control by, 410
particle size, determination, 8
phytocidal action, 1149
reports of Committee on Standardization of Tests, 624, 627, 633
seed-protectant, organic vs. inorganic, 9
slide-germination method of assay, 627
seed-treatment, for cereal diseases, 1064
for peas, 1098
sodium nitrite for damping-off, 1110
soil-, for Fusarium oxysporum cubense, 398
on soy bean, effects on emergence, stand and yield, 1113
standardization of tests, committee report, 424
sulphur, effect on: apple growth and yield, 2, 56
apple scab, 1119
particle size, infection control vs., 533
for Phymatotrichum sclerotia, 1205
sulphur (elemental), effects on white clover composition by rust and, 401
synergism and antagonism between, significance, 18
terms, definitions, 624
tetrachloro-para-benzoquinone (see Spergon)
tetra - methyl - thiuram - disulphide (see Thiosan; Jap Beetle Spray)
Thiosan (see Thiosan)
vapor action of dust-, on seed-borne fungi of cotton, 431
for vascular diseases, internal therapy, 16
volatile vs. nonvolatile, for oats smuts, 1112
water-soluble protectant, disodium ethylene bisdithiocarbamate as, 1095
wood preservative-, new adaptations, 17
zinc sulphate-lime effect on protective value, 1113
Fungistatic action, of 8-hydroxyquinoline, mechanism, 1121
Fusarium spp., on cereals in Manitoba, 1111
on cotton vs. sweet potato vs. tobacco, 478
in deciduous nursery tree root rot and control, 656
mushroom disease role, 1090
pea infection by soil-borne, soil and seed treatments for, 1098
potato charcoal rot association, 1120
wheat "black point" role 1112
bulbigenum var. batatas, on sweet potato, 469
var. lycopersici, host-parasite relations of mild vs. virulent forms, 186
inhibition by tomato-plant sap, 1111
pathogenicity, variations and factors in, 175
toxic effects of extracts of mild vs. virulent, 1004
toxin in sap of infected tomato, 4, 126
moniliforme, cotton hypocotyl infection, histopathology, 666
fungicidal vapor effects on, 431
oxysporum, physiologic races, 481
in wilt of cotton vs. sweet potato vs. tobacco, 469
f. conglutinans, on cabbage, host nutrition role, 15
cubense, as influenced by soil and chemical mixtures, 398
lysis by Actinomyces sp., 403
soil application of mercurials as affecting banana plants vs., 835
var. nicotianae, on tobacco, 469
**retusum** n. sp., tomato vascular wilt due to, 957
vasinfectum, on cotton, pathogenicity, 469
root knot and, soil amendment effects, 10

Genetics (see also Breeding; Mutation)
bean-, mosaic symptoms expression, 16
fungus-, interspecific hybridization, 584
segregation in basidiospores, 3
Sorosporium, 569
Sphacelotheca, 569
Ustilago, 749, 943
Venturia, 11, 19
of klendusity to tobacco mosaic, 19, 691
Germination, spore-, method for Agaricus, 530
Gibberella zeae, on corn, inoculation method, 16
GLICK, D. P., (245)
**Gloeocercospora sorghi** n. gen. and n. sp., sorghum zonate leaf spot due to, 225

Gloeodes pomigena, on pear, Elgetol as eradicant, 1117
Gloeosporium sp., in tobacco anthracnose, 15
necator, G. venetum vs., taxonomic status and Rubus anthracnose role, 1115
venetum, G. necator vs., taxonomic status and Rubus anthracnose role, 1115
Glomerella cingulata, on lupines, 249
on tomato, control by Fermate, 1120
gossypii, cotton hypocotyl infection, histopathology, 666
fungicidal vapor effects on, 431
Glomerularia lonicerae, life history and pathogenicity, 4
on Lonicera, reactions of spp. and vars. to, 4
GOODWIN, M. W., 1114
Gossypium spp., Puccinia stakmanii (exp.) on, 387
Rhizoctonia on, 155
GOTTLIEB, DAVID, 4, 126, (619), 724, 1111, (1113)
GOULD, C. J., 4
Graft disease, apple Rhizoctonia, 255
Grape, boron deficiency in, 940
copper fungicides for, disease control vs. spray injury, 13
Grasses, Erysiphe graminis pathogenicity to Hordeae, 5
fine turf-, disease control tests on, plot technique, 528
forage-, smuts of cereals on, reactions to, 910
fungus seedling blight and root rot, 234
Gloeocercospora sorghi on, 221
Kentucky blue- (see Poa pratensis)
redtop- (see Agrostis alba)
smuts, chlamydospore germination, temp. effects, 7
culture of fungi, 7
Sorosporium and Sphacelotheca, genetical, cytological and cross inoculation study, 569
Typhula snowmold, general study, 1157
GRAVES, ARTHUR HARMOUNT, 18
GREANEY, F. J., 4, 1064, 1111
Green stinkbug, Nematospora phaseoli association, 1209
GRIES, GEORGE A., 1111, 1112
GRIFFITH, ROBERT B., 745
Growth, fungus-, reflector scale for measuring, 619
Growth substances, effect on rust fungi, 724
pathological growths and symptoms due to, comparisons, 1119
wax bean blossom drop reduced by, 1109
GRUENHAGEN, R. H., (11), 1112
Guignardia bidwellii, on grape, copper fungicides for, control vs. spray injury, 13
Guttation fluids, effects on: foliage, 1110
pesticides, 1110
Gymnosperms, mycorrhizas of, in Colo., 146
Gymnosporium juniperi-virginianae, fungicidal control, by Fermate, 5
particle size vs., 533
sporidia, preservation of viability by quick freezing and storage, 612
HAHN, GLENN GARDNER, 341
HAMILTON, J. M., 5, 533, 612, (683)
HAMM, PHILIP, 619
HANSING, E. D., 1112
HANSON, E. W., 1112
Haplosphaeria deformans, stamen blight of blackberry and dewberry due to, distribution and general study, 136
HARDISON, JOHN R., 5
HARE, W. W., 15
HARROLD, THOMAS J., 666
HART, HELEN, 335, 484, (724), 1113
HARTER, L. L., (16)
Heartwood, western red cedar-, effect of extracts on wood-destroying fungi, 517
Heat injury, effect on potato soft rot in transit, 1117
Heat treatment, for fungi on seeds, 333
Hedera helix, Sphaceloma leaf scab, 1109
Helminthosporium spp., on cereals, in Manitoba, 1111
on corn, inoculation method, 16
wheat "black point" role, 1112
avenae, on oats, seed treatments for, 1064
sativum, grass seedling blight and root rot due to, 238
on seed of barley and wheat, in Canada, 4
seed treatments for, 1064
wheat kernel smudge due to, varietal reactions, 4
turcicum, dent corn leaf blight due to, 407
on inbreds and hybrids, 18
HENRY, A. W., 332
HENRY, BERCH W., 18
HENRY, WENDELL D., (1119)
Heterodera marioni, control, by chloropicrin fumigation of soil, 1166
by sodium nitrite soil treatments, 1110, 1111
on cotton, Fusarium and, soil amendment effects, 10
on tomato roots, treatment for greenhouse control, 6
HEUBERGER, J. W., (13), 18, (1095), 1113, (1190)
Hibiscus sp., Phytomonas leaf spot, 82
Puccinia stakmanii (exp.) on, 387
HILBORN, M. T., 45
HILDEBRAND, E. M., 6, 18, 167
HINES, LEE, (884)
HOFFMASTER, D. E., 1113
HOLMES, FRANCIS O., 19, 691, 1114
HOLTON, C. S., 169, 732, (910)
Homalocladium platycladum, Erysiphe powdery mildew on, 745
HOOKER, W. J., 15
HOPPE, PAUL E., 528, 602
HOPPERSTEAD, S. L., 1114
Hordeum jubatum var. caespitosum cereal smuts on, reactions to, 915
HORNE, W. T., (719)
HORSFALL, JAMES G., (16), (18), (1095), 1114, 1121
HOWARD, F. L., 6, 12, 1114, 1115
HUMPHREY, H. B., 531
Hybridization, fungus, interspecific, 584
8-Hydroxyquinoline, mechanism of fungistatic and bacteriostatic action, 1121

Hyperparasitism, in oomycetes, 287
Hypoxylon pruinatum, on aspen, life history vs. pathogenicity, 1112

IMMER, F. R., 253
Immunity, from virus, acquirement in tobacco, 598
Inactivators, "non-toxic," effect on viruses of plants, 675
Inheritance (see under Genetics)
Insecticides (see also Pesticides)
new-type spray equipment for, efficiency, 1114
Insects, elm-, Beauvaria bassiana on, distribution in U. S. A., 76
Insects as vectors of: carnation mosaic (?), 826
cherry yellows by leaf hoppers, 466
Nematospora phaseoli, green stinkbug association, 1209
phlox "breaking," by aster yellows via leaf hoppers, 741
pineapple yellow spot by thrips, 408
potato purple-top wilt, by aster yellows via leaf hoppers, 16
potato yellow dwarf, by Aceratagallia sanguinolenta, 17, 363
by Agallia constricta, 17
specificity of leaf hoppers to vars. of, 1110
virus varieties, specificity, 17
IRISH, C. F., (1196)
Ivy, English (see Hedera helix)

Jap Beetle Spray, in preharvest cherry sprays, 683
JENKINS, ANNA E., (1), 6, 168, 404, (1109), 1115
JOHANN, HELEN, 526
JOHNSON, A. G., (169)
JOHNSON, E. M., (3), (210), (1120)
JOLIVETTE, J. P., (778)
Juglans spp., juglone fungicide from, 1112
nigra, root rot of nursery trees, causes and control, 656
Juglone, fungicidal value, 1112

KEIL, H. L., (1115)
KEITT, G. W., 6, 19, 449, 1115, 1212, 1213
Kernel smudge, wheat-, 4
KERNKAMP, M. F., (943)
KING, C. J., (382)
KING, T. H., (943)
KLEIN, HAROLD P., (314)
Klendusity, heritable, to tobacco mosaic in Lycopersicum chilense and hybrids, 19, 691
KLIGMAN, A. M., 1090
Knobby bark, citrus-, virus (?) origin, 862
KOCH, KARL, 19
KOTILA, JOHN E., 6
KOUBA, T. F., (11)
KREITLOW, K. W., 7, 707, 1055
KREUTZER, W. A., 245
KUNG-HSIANG LIN (see LIN)
KUNKEL, L. O., 19

LANGFORD, M. H., (19)
Larkspur, rocket-, bacterial bud and stem rots, factors influencing, 806
varietal reactions, 810
LARSON, R. H., 1116, 1216
LEACH, J. G., (14), 339, (406), 747, 1116, (1119), 1209
LEACH, L. D., 7
Leaf blight, sugar beet Corticium, 6
Leaf blotch, oats Helminthosporium, 1064
Leaf curl, peach-, 259
Leaf disease, Kentucky bluegrass-, Septoria-induced, 745
Washington palm bacterial (new), 1201
Leaf hoppers, as vectors of: aster yellows virus to phlox, 741
cherry yellows, 466
potato yellow dwarf, 363
potato yellow dwarf vars., 1110
Leaf scab, English ivy Sphaceloma, 1109
Leaf spot, apple frog-eye-, 1116
cantaloupe physiological-, root aeration role, 162
cherry-, 9, 1115
dandelion (Russian) bacterial, 959
hibiscus Phytomonas, 82
oats Pseudodiscosia, 165
plum-, noninfectious heritable disorder of Beaty var., 1101
raspberry "Septoria," Sphaerulina rubi (new) causing, 986
rice Cercospora, 70
sorghum zonate-, 220
of Terminalia arjuna, fungus etiology (?), 404
tobacco bacterial, 3, 1120
tobacco Cercospora, 354
LEAR, BERT, 1118
Leaves, area estimation technique, on potato, 922
on sugar beet, 1071
copper absorption by, fungicidal protection role, 1151
fungicide redistribution on, significance, 5
guttation-fluid effects on, 1110
LEHMAN, S. G., 431
Lentinus lepideus, as influenced by heartwood extracts of western red cedar, 518
LEONARD, O. A., (10)
Lettuce, Rhizoctonia damping-off, sodium nitrite soil treatments for, 1110
LEVINE, MICHAEL, 8
LEVINE, M. N., 1118
LEWIS, RALPH W., 1116
Leytosan, fungicidal action of vapor, 445
Lightning injury, to cotton, types, symptoms, and effects, 150
LIMBER, DONALD P., 80
LIN, KUNG-HSIANG, 394
LINCOLN, F. B., (255)
LINFORD, M. B., 408
Liriodendron tulipifera, root rot of nursery trees, causes and control, 656
Locust, black- (see Robinia pseudoacacia)
LOEGERING, W. Q., (12), (253), (884), (1118)
LOJKIN, MARY E., 8
Lonicera spp., Glomerularia lonicerae on, reactions to, of spp. and vars., 4

LOWTHER, C. V., (406), (1116)
Lupinus spp., Botrytis cinerea on, 319
angustifolius, anthracnose, 249
Lumber (see Wood)
Lycopersicon spp., Alternaria blight vs. "physiological" maturity, 1
chilense, tobacco mosaic klendusity (heritable) in hybrids and, 19, 691
Lygus sp., blossom drop in wax bean associated, 1109
LYLE, J. A., 1116
Lysis, of Ustilago promycelia, heritable, 751

MCCALLAN, S. E. A., (15), 19, 424
MCCUBBIN, W. A., 422
MCDONNEL, A. D., (1114)
MCFARLANE, JOHN S., (1104)
MCKINNEY, H. H., 8, 300, 551, 1045
MACLACHLAN, J. D., 8
MCLAUGHLIN, J. H., (1113)
MCLEAN, RUTH A., 354
MCNAMARA, H. C., (10)
MCNEW, GEORGE L., 9
MACHACEK, J. E., (4), (1111)
MACK, G. L., (5), 8, (533)
Maclura pomifera, root rot in nursery, causes and control, 656
Macrophomina phaseoli, dry rot of crops due to, in southern U. S. A., 1113
Macrosporium solani, on tomato, spray control, factors influencing, 9
Macrosteles divisus, as vector of: aster yellows, to phlox, 741
to potato, 16
MADER, E. O., 1134, 1185
Magnolia grandiflora, Sphaceloma scab (new) on, 6
Malignancy, in animal vs. plant tumors, 98
Malva parviflora, Puccinia stakmanii (exp.) on, 387
Mango, Elsinoë scab, 1
MANNS, T. F., (1113)
Manures, effect on Fusarium-root knot-attacked cotton yields, 10
Maple, red- (see Acer rubrum)
Verticillium infection, internal therapy, 16
Marmor tabaci, cyanide effects on synthesis, 77
klendusity to, in Chilean tomato and hybrids, 691
vastans, clover leaf hopper transmission relations, 363
varieties, vector specificities for, 17
MARTEN, E. A., 406
MARTIN, W. J., 75, 569, (943)
Melilotus alba, Ditylenchus dipsaci strain on white clover, alfalfa and, 525
Melons, honeydew, anthracnose as influenced by packing methods, 245
Mercurials, fungicidal (see under Fungicides)
MEREDITH, CLIFFORD H., 398, 403, 835
Microorganisms, toxicity of crop residues in relation to, 1111
Mildew, bean powdery-, 1148
cucumber powdery-, 1147
delphinium Sphaerotheca powdery-, 832
grape downy-, 13
grape powdery-, 13
grass powdery-, 5
Homalocladium platycladum powdery-, 745
Lima bean downy-, 2
rose powdery-, 1097
tobacco downy-, 101
MILLER, HAROLD J., 9, 899
Millet, Ustilago crameri on, pathogenesis, 1129
MILLS, P. J., (713)
Minuor **rubrorum** n. sp., raspberry decline virus, 161
Mirid, tomato "red ring" due to, 613
MITCHELL, J. E., (619), (1113)
Mites, mushroom "bacterial pit" role, 1092
Mitochondria, virus evolution from derivatives and, chemical evidence, 766
cytological and genetical evidence, 637
Mitra, Manoranjan, biographical note, 429
Mold, cherry gray-, 683
Moniliochaetes infuscans, on sweet potato, fungicidal control, 410
MOOK, PAUL V., 76
MOORE, J. DUAIN, (6), (1115), (1212), (1213)
MOORE, M. B., 423, 1117
MOORE, W. D., 797, 1176
Morus alba f. tatarica, root rot in nursery, causes and control, 656
Mosaic, bean, productivity and quality of resistant snap varieties, 778
virus-4, symptom expression, genetics of, 16
carnation, symptoms, virus nature, transmission and distribution, 823
cucumber, virus, passage from cion to stock, contact period effects, 818
sensitivity to inactivators, 675
elm (American) graft-transmissible, 1196
pea bean-, new, 1215
Plantago virus, reactions of resistant tobaccos to, 564
potato X-, foliar mottle and necrosis in Chippewa var. associated with strain of, 1116
sugar cane, internal breakdown associated, 3
re-infection of recovered plants, 713
virus disappearance from recovered plants, 713
tobacco, genotypes resistant to, 300
klendusity to, in Chilean tomato and hybrids, 691
N and N′ factors in relation to, 14
reactions of resistant vars. and spp. to strains and mutants, 551
tomato derivatives escaping, 19, 691
virus, cyanide effects on synthesis, 77
origin and relationships, 219
sensitivity to inactivators, 675
virus-1, acute and chronic phases and factors influencing, 1052
virus-protein, colorimetric determination, 8

yellow-, acute and chronic phases and factors influencing, 1045
variegation resembling, in snap beans, 779
"white," reactions of resistant tobaccos to, 563
yellow virus, type-B, reactions of resistant tobaccos to, 564
type-C, reactions of resistant tobaccos to, 565
Mosaic spot, of figs, 719
MOSES, C. S., (18)
Muhlenbergia arenacea, Puccinia stakmanii (exp.), on, 387
MULLER, A. S., (1086)
Mummy disease, mushroom-, description and etiology, 1090
MUNCIE, J. H., (1071)
Mushroom, diseases, 1090
resistance to, mechanism, 13
fructification and production, inhibitory factors, 1134
truffle disease, control by ammonia, 13
Musivum tabaci var. **plantaginis** n. comb. (= Plantago virus), 219
Mutation (see also Breeding, Genetics)
in Ustilago, 760
in Venturia, 11, 19
Mycology, Saccardo's Sylloge Fungorum, note on reprinting of, 635
Mycorrhizas, forms and hosts in Colorado, 144
Mycosphaerella rubi, as perfect stage of Septoria rubi, 1002

NATIONAL RESEARCH COUNCIL, Division of Biology and Agriculture, committee report on, 420
Necrosis, apple internal bark-, boron vs., 1
Necrotic-cavity corky bark, citrus-, virus (?) origin, 860
Nemacides, fumigant, for root knot, 1118
chloropicrin as, 1166
sodium nitrite, for root knot, 1110, 1111
Nematodes (see also Ditylenchus; Heterodera)
Nematospora phaseoli, on Lima bean, association with green stinkbug, 1209
New Improved Ceresan, fungicidal action of vapor, 431
New Improved Semesan, Jr., corn seed treatment by, 602
NEWHALL, A. G., 9, 61, (1118)
NEWTON, MARGARET, 10
Nezara hilaris, Nematospora phaseoli association, 1209
Niacin, effect on rust fungi, 724
Nicotiana spp., Phytophthora parasitica nicotianae resistance in, 403
virus-strain reactions of resistant, 551
glutinosa, N factor in, effect on mosaic expression, 14
rustica, potato yellow dwarf on, local lesion factors, 1121
tabacum, chromosomes, N and N′ factors, relative positions, 14
Nicotiana virus-1, reactions of resistant tobaccos to strains and mutants, 551
in tobacco, acute and chronic phases and factors influencing, 1052
genotypes resistant to, 300
Nicotiana virus-6, reactions of resistant tobaccos to, 565
Nicotiana virus-12, reactions of resistant tobaccos to, 565
NIEDERHAUSER, JOHN S., 959
NIELSEN, L. W., 1117
Nucleoproteins, plastid-, virus nucleoprotein evolution from, 774
properties, 772
Nursery trees, deciduous, root rot and control in, 656

Oaks, Chalara sp. causing rapid dying, 18
bur- (see Quercus macrocarpa)
Oats, Alternaria on seeds, storage temp. effects, 333
covered smut, seed treatment by Spergon, 332
Oats, crown rust pathogenesis, cytochemistry, 531
Pseudodiscosia leaf spot, in Turkey, American form compared, 165
seed-borne diseases, seed treatments for, 1064
smuts, physiologic races, exptl., 14
seed treatments for, volatile vs. nonvolatile fungicides, 1112
stem rust, races in U. S. A., 1118
reactions as influenced by vitamins and growth substances, 724
Oil, mineral-, in culture maintenance, 330
Olive, boron deficiency in, general study, 933
Onion, nematode injury, pathogenesis, 61
smut, control by Catex, 9
Oomycetes, hyperparasitism among, 287
Oospores, Pythium, Trichothecium parasitizing, 232
Orange (see also Citrus)
boron deficiency in, 939
Orchids, Erwinia soft rot, 80
mycorrhizas of, in Colorado, 147
Ornamentals, Verticillium-free clones, establishment, 3
Orobanche ludoviciana, tomato parasitized by, 257
OSBORN, JOHN H., (372)
Overgrowths, plant-, chemical factors in, 1119

Palm, Washington-, bacterial leaf disease (new), 1201
PALMITER, D. H., (5), (533), 683, 1117
Panama disease, banana-, 835
Panicum spp., smuts of, cross inoculations, 571
Panus stipticus, cordwood decay by, calorific and chemical study, 46
Para Bacca, fungicidal action of vapor, 445
Parasitism (see Antagonisms, etc.)
Particle size, determination in fungicides, 8
Pasmo disease, flax-, 1113
Pasture plants, tobacco leaf-spot bacteria on roots of, 1120
Pathogenesis, toxin role, in tomato Fusarium wilt, 126

Pea, diseases, survey in Wis., 15
seed protectants for, 14
seed and seedling diseases, soil and seed treatments for, 1098
Peach, Armillaria root rot in eastern U. S. A., 812
diseases, control by Fermate, 962
fungicides on, field testing technique, 13
leaf curl, early records in U. S. A., 259
Sclerotinia blossom blight, temp. and humidity effects, 15
suture spot, new fruit disease (cause unknown), 167
yellow-red virosis, symptoms, techniques for localizing and intensifying, 18
virus, fungus toxins compared, 20
Peanut, Macrophomina dry rot, 1113
Pear, sooty blotch, Elgetol as eradicant, 1117
PENNY, J. S., (1090)
Permeability, in rust resistance mechanism, 14
Peronospora spp., species characterization, bases, 112
nicotianae, status, 106
tabacina, characterization, morphology vs. pathogenicity in, 109
distribution, 108
Pestalotia disseminata, on Terminalia arjuna, 404
Pesticides, guttation-fluid effects on, 1110
PETURSON, B., (10)
Phlox, annual, aster yellows infection via leaf hoppers, 741
Phlox drummondii, aster yellows infection via leaf hoppers, 741
Phoma terrestris, corn pink-root infection due to, 526
Phomopsis sp., on Terminalia arjuna, 404
vexans, on eggplant, Fermate-talc dust control, 1117
Photoperiodism, effect on wheat bunt, 240
Phyllosticta sp., on Terminalia arjuna, 404
Phymatotrichum omnivorum, sclerotia, inhibition by S autoclaved with soil, 1205
Physalis subglabrata, tobacco etch on, 1114
Physalospora tucumanensis as perfect stage of Colletotrichum falcatum, 2
Physiological disease, cantaloupe leaf spot, root aeration role, 162
"Physiological" maturity of tomato, 1
Physiological races of: Cercospora oryzae, 70
Erysiphe graminis, 5
Fusarium oxysporum, 481
Puccinia graminis avenae, 724, 1118
Puccinia graminis tritici, 253, 335, 485, 724, 884
Urocystis agropyri, 919
Urocystis tritici, 169
Ustilago, 14
Ustilago nigra, 324
Phytolacca decandra, extract, effect on viruses of plants, 675
Phytomonas spp., growth in synthetic medium as taxonomic aid, 314
poinsettiae, greenhouse dissemination, 10
sepedonica, culture maintenance, 330
isolation, differential medium for, 406
solanacearum, nitrogen utilization, forms and concentrations, 14
stewartii, dent corn leaf blight due to, 407
syringae, hibiscus leaf spot by, 82
tolaasi, mushroom blotch due to, 1093
tumefaciens, absence from secondary tumors, 85
pathological growths by chemicals resembling those by, 1119
**washingtoniae** n. sp. leaf disease of Washington palm due to, 1203
phytophthora (Erwinia), 806
Phytophthora infestans, on potato, severe infection on Sebago var., 1104
on tomato fruit, effect on leaf blight spray control, 9
parasitica nicotianae, resistance of Nicotiana spp. to, 403
Picric acid crystals, fungicidal action of vapor, 445
PIERCE, E. C., 162
Pierstorff, Arthur Lewis, biographical note, 430
PINCKARD, J. A., 10
PINE, LEO, 1201
Pine(s), hard-, Diplodia pinea on, pathology and distribution, 1018
red- (see Pinus resinosa)
soft-, Diplodia pinea on, significance, 828
Sphaeropsis malorum on, significance, 828
white-, (See Pinus strobus)
Pineapple, yellow spot, plant spacing vs. incidence, 408
wind-blown vector, 410
Pink roots, on corn, Phoma terrestris causing, 526
Pinus spp., Diplodia blight of nursery and mature trees, 390
Diplodia pinea on, pathology and distribution, 1018
significance, 828
diseased regions in, determination by dyes, 20
nonconducting tissues, determination by dyes, 20
Sphaeropsis malorum on, significance, 828
resinosa, damping-off in nursery, seed dust-soil treatment for, 11
strobus, blister rust on, red currant role, 341
selection for resistance, 11
PIRONE P. P., 10
Pit, mushroom "bacterial," mite role, 1092
PLAKIDAS, A. G., 1117
Plane tree, London- (see Platanus acerifolia)
Plantago virus, in burley tobacco, 210
origin and relationships, 219
reactions of resistant tobaccos to, 564
in tomato (exp.), 216
Plants, sap, anaerobic collection method, 129
anatomy, book review, 623

coacervates in cells, origin and artificial production, 739
residues, effect on root diseases, 1111
Plasmopara viticola, conidia, preservation of viability by quick freezing and storage, 613
on grape, copper fungicides for, control vs. spray injury, 13
Plastids, virus evolution from nucleoproteins of, chemical evidence, 766
Platanus acerifolia, bacterial rot of winter-injured wood, 963
Polyporus infection of winter-injured wood, 963
Plectospira myriandra, Pythium attacked by, 291
Plum, noninfectious heritable leaf-spot and shot-hole of Beaty var., 1101
Plum-cherry hybrids, Sclerotinia fructicola from, variation in single-spore isolates, 1118
Poa pratensis, Septoria oudemansii leaf infection, 745
stripe smut, chlamydospore germination, temp. effects, 7, 1055
host development vs. temp., 1055
Poinsettia, bacterial infection, greenhouse dissemination, 10
poinsettiae (Phytomonas), 10
Polyporus spp., black cherry rots due to, general study, 984
cordwood decay by, calorific and chemical study, 46
as influenced by heart wood extracts of western red cedar, 518
versicolor, in winter-injured plane tree wood, 963
Pome fruits, Armillaria root rot, in eastern U. S. A., 813
Poria spp., black cherry rots due to, general study, 984
xantha f. crassa, as influenced by heartwood extracts of western red cedar, 518
PORTER, RICHARD, P., 1117
Potassium cyanide, effect on tobacco virus synthesis, 77
Potato, Andean fungus disease, preliminary report, 1086
Potato, bacterial ring rot, culture of pathogen, 1118
maintenance, 330
general study of organism, 1032
isolation of organism, medium for, 406
survival of pathogen in storage, 1110
charcoal rot, Sclerotium bataticola causing, 1120
early blight, zinc sulphate-lime effect on fungicidal control, 1113
foliar mottle and necrosis of Chippewa-, potato X-virus strain associated, 1216
late blight, severe on Sebago var., 1104
leaf area, estimation technique, 922
leaf roll, effect on leaf area increase, 932
Macrophomina dry rot, 1113
purple-top wilt, aster-yellows virus causing, 16
transmission by Macrosteles divisus, 16
ring spot, virus, sensitivity to inactivators, 675
seed-, virus disease control in, 19
soft rot in transit of early, sunscald role, 1117
southern (Corticium) blight, description and soil moisture relations, 615
X-virus, foliar mottle and necrosis in Chippewa var. associated with strain of, 1116, 1216
yellow dwarf, on Nicotiana rustica, local lesion factors, 1121
vector specificities for vars. of, 17, 1110
vector transmission relations, 363
Preservatives, wood-, new adaptations, 17
PRESLEY, JOHN T., 382
PRICE, W. C., 10, 586
Prunus (see also Apricot; Peach; Plum; Stone fruits)
cherry yellows on, 459
americana, root rot in nursery, causes and control, 656
cerasus, Sclerotinia laxa imperfect stage on, in Wis., 1212
serotina, cull from decay, causes and factors influencing, 965
decays, managements vs., 977
Pseudobalsamia microspora, on mushrooms, control by ammonia, 13
Pseudodiscosia avenae, on oats in Turkey vs. America, 165
Pseudomonas spp., phytopathogenic, growth in synthetic medium, as taxonomic aid, 315
fluorescens, mushroom mummy disease role, 1090
Pseudomycorrhizas, hosts in Colo., 144
Pseudoperonospora cubensis, fungicidal action on, factors, in, 1147
Psorosis, citrus-, comparative symptomatology of forms of, 837
wood alterations vs. tree deterioration, 865
Puccinia carduncelli, hosts, 792
carthamni, distribution, 789, 791
on safflower, 789
coronata, on oats, pathogenesis, cytochemistry, 531
graminis avenae, as influenced by vitamins and growth substances, 724
races in U. S. A., 1118
secalis, on barley, varietal reactions, 253
tritici, on barley, varietal reactions to physiologic races, 253
as influenced by vitamins and growth substances, 724
physiologic races, population trends (U. S. A., 1930–41), 884
race 15-b, on Triticum timopheevi, 335
resistance to, mechanism, 14
testing for, 897
on wheat, browning reaction, host vars. and rust races vs., 484
regional spread from barberries (1942), wind role, 12

kentrophylli, synonym of P. carthamni (?), 791
**stakmanii** n. sp., on cotton, 385
distribution, hosts and general study, 385
verruca, on safflower, 794
Punica granatum, Sphaceloma on, 1
Purple-top wilt, potato-, aster yellows causing, 16
tranmission by leaf hoppers, 16
Pyrax AAB-Fermate mixture, tomato early blight control by, 1119
Pythiogeton autossytum, Pythiums attacking, 289
Pythium spp., antibiotic relations among, 287
oomycetes attacking, 287
pine damping-off by, seed dust-soil treatment for, 11
acanthicum, Pythiums parasitized by, 287
graminicolum, grass seedling blight and root rot by, 238
oospores, Trichothecium (new) parasitizing, 232
myriotylum, distribution, hosts, life history and pathogenesis, 261
oomycete parasites of, 289
oligandrum, Pythiums parasitized by, 287
**ostracoides** n. sp., fungi growing on, 292
on wheat roots, cyto-morphology, life history, and pathogenesis, 275
periplocum, Pythiums parasitized by, 287
ultimum, Aloe variegata root rot by, control by hot water, 736
cotton damping-off by, conditions favoring, 607
pre-emergence damping-off by, measure of, seedling germination rate/fungus growth rate as, 7
seed decay by, protectant fungicides for, 9
water-soluble fungicide for, 1097

Quercus spp., Chalara sp. causing rapid dying, 18
macrocarpa, root rot of nursery trees, causes and control, 656

RADER, WILLIAM R., (9)
RAND, FREDERICK V., 419
RANDS, R. D., 1107
Rasp leaf, cherry-, virus nature, distribution and description, 620
Raspberry, "Septoria leaf spot," Sphaerulina rubi (new) causing, 986
virus decline, symptoms, transmission and control, 156
RAWLINS, T. E., 623
RAY, W. WINFIELD, (17), 51, (1113)
"Red ring" of tomato stems, insect-induced, 613
Redtop (see Agrostis alba)
REED, HOWARD S., 739
Reflector scale, for fungus growth measurements, 619
Regulatory work and foreign plant diseases, committee report, 422
REINKING, O. A., (8)
Reports, American Phytopathological Society (and Divisions) (see under AMERICAN . . .)
Residues, crop-, effect on root diseases, 1111
Resistance, barley, to stem rust races, 253
bean, to mosaic, 778
to mosaic virus-4, 16
cereals, to rust, role of vitamins and growth substances, 724
chestnut, to blight, 18
corn hybrids, to Helminthosporium leaf blight, 408
delphiniums, to bacterial crown, stem and bud rots, 810
grasses, to Erysiphe graminis, 5
Lonicera, to Glomerularia lonicerae, 4
mushrooms, to disease, mechanism, 13
Nicotiana spp., to Phytophthora parasitica nicotianae, 403
pine, to blister rust, 11
red currant, to blister rust, 346
to rust fungi, mechanism, 14
tobacco, to Cercospora leaf spot, 358
to Nicotiana virus-1, 300
real vs. apparent to Cercospora leaf spot, 358
to viruses, 551
tomato, to tobacco mosaic, 691
wheat, to stem rust, 897
Reviews, book, anatomy of plants, 623
forest-tree seeds, 338
REYNARD, GEORGE B., 613
Rhizoctonia spp., damping-off, sodium nitrite soil treatments for, 1110
pine damping-off by, seed dust-soil treatment for, 11
on flax, boron effects, 1113
bataticola, on lightning-injured cotton, 154
solani, alfalfa root canker due to, general study, 1081
basidiospores, segregation in, 174
on cotton, lightning-injured, 154
seed dusting vs. seedling infection, 51
culture variations in single-basidiospore isolates, 3, 171
damping-off by, measure of, seedling germination rate/fungus growth rate as, 7
solani (?), layer and graft infection on apple, 255
Ribbon bush, Erysiphe powdery mildew on, 745
Ribes spp., distribution, measuring local, 4
red currant vars. and, blister rust relations, 341
Riboflavin, effect on rust fungi, 724
RICE, PAUL L., (1114)
RICHARDS, B. LORIN, 1215
RICHARDS, M. C., (1)
RIEMAN, G. H., 1104
RIKER, A. J., 11, (1109), (1119)
Ring rot, potato bacterial, 406, 1110
Ring spot, tobacco, virus, cyanide effects on synthesis, 77
passage from cion to stock, contact period effects, 818
sensitivity to inactivators, 675
RISCHKOV, V. L., 950
ROBERT, ALICE L., (333)

Robinia pseudoacacia, root rot on nursery stock, causes and control, 657
RODENHISER, H. A., 240
Root diseases, crop-residue effects on, 1111
Root knot (see Heterodera marioni)
Root rots, of Aloe variegata, Pythium-induced, 736
in deciduous nursery trees, 656
fruit tree Armillaria, 812
grass fungus seedling-, 234
by oomycetes, antibiotic relations among pathogens, 261
Phymatotrichum, 1205
Roots, aeration, cantaloupe physiological leaf spot role, 162
tobacco leaf-spot bacteria on various plant-, 1120
Rose, black-spot, incidence vs. mineral nutrition, 1185
flower production of own-rooted, mineral nutrition effects, 1185
water-soluble fungicide for black spot and powdery mildew, 1097
ROTH, F. L., (11)
Rots, black cherry fungus-, 965
cherry brown-, 683
corn ear-, Diplodia macrospora in, 528
corn ear-, and stalk-, 16
delphinium crown-, stem-, and bud-, 806
grape black-, 13
Macrophomina dry-, 1113
orchid Erwinia soft-, 80
pea (soil-borne), 1098
peach brown-, 962
plane tree (London) bacterial, 963
potato bacterial ring-, 330, 1032, 1118
potato Sclerotium charcoal-, and associates, 1120
potato soft-, 1117
Pythium myriotylum soft-, 261
Sclerotinia brown-, 546
stone fruit Sclerotinia brown-, 506, 1120, 1212
sugar cane Colletotrichum red-, 2
wood-, fungus-induced, 17, 45, 517
of hardwoods, 45
Rubus spp., anthracnose, etiology and history, 1115
Rubus virus-8 (new), raspberry decline due to, 161
Ruffled leaf, cherry- (see Rasp leaf)
RUPERT, J. A., (14)
Rusts, apple cedar, 5, 533, 1118
barley stem-, 253
bean-, 1147
beet-, 10
carnation-, 1191
cotton Puccinia (new), 385
flax-, 1113
as influenced by vitamins and growth substances, 724
oats crown-, 531
oats stem-, 724, 1118
peach Tranzschelia, 962
resistance to, mechanism, 14
safflower-, 789
wheat stem-, 12, 484, 724, 884
on Triticum timopheevi, 335
white pine blister- (see Cronartium ribicola)
Rutabagas, water-core in, control by borax in soil vs. spray, 8
Rye, ergot epidemic production on, technique, 1116
RYKER, T. C., 70

SACCARDO'S SYLLOGE FUNGORUM, note on reprinting of, 635
SACCO, PAUL, (1109)
Safflower, rusts of, 789
Sanoseed, fungicidal action of vapor, 434
Sap, plant-, anaerobic collection method, 129
effect on tomato wilt fungus, 1111
Scab, apple-, 5, 6, 11, 19, 56, 533, 1097, 1114, 1118, 1119, 1192
English ivy leaf-, Sphaceloma causing, 1109
magnolia Sphaceloma (new), 6
mango Elsinoë, 1
violet Sphaceloma, 168
Sclerotia, Phymatotrichum, inhibition by S autoclaved with soil, 1205
Sclerotium rolfsii, soil- sterilizing agents for, wetting agent effects on, assay, 12
Sclerotinia fructicola, on cherry, organic materials for pre-harvest spray, 683
from cherry-plum hybrids, single-ascospore isolates, variation in culture, 1118
conidia, preservation of viability by quick freezing and storage, 613
fungicidal control, particle size vs., 546
on peach, control by Fermate, 962
on stone fruits, temp. and humidity effects on blossom blight, 15
laxa, chlorophenates as eradicant sprays for, 1120
imperfect stage on Prunus cerasus in Wis., 1212
on stone fruits, eradicant sprays for, 506
Sclerotium bataticola, dry rot of crops due to, in southern U. S. A., 1113
potato charcoal rot due to, 1120
rolfsii, sclerotia, soil-sterilizing agents for, wetting agent effects on, assay, 12
SCOTT, C. EMLEN, 933, 962
Scurf, sweet potato-, 410
Seed treatments, cereals, for seed-borne diseases, 1064
by Spergon, 332
cereal and vegetable, report of Coordination-of-Research Committee, 423
corn, by mercury vs. non-metallic dusts, 602
cotton, for damping-off, 51
effect on disease control and yields, 17
by fungicidal vapors, 431
flax, seed injury vs., 1117
by Spergon, 332
Lima bean, 3
oats, for smut, volatile vs. nonvolatile fungicides, 1112
pea, for rots (soil-borne), 1098
protectant, by organic vs. inorganic fungicides, 9
for vegetables, 14
soy bean, effect on emergence, stand and yield, 1113
tomato, for Alternaria solani, 797

Seed-borne diseases, Alternaria zinniae, 379
carrot Cercospora blight, 119
cereal, in Manitoba, 1111
seed treatments for, 1064
pea, control, 1098
forest trees, book review, 338
Seeds, forest tree-, book review, 338
fungi on cereal-, heat treatment for, 333
Segregation, in basidiospores, 3
Semesan Bel, sweet potato scurf control by, 410
sepedonica (Phytomonas), 330, 406
sepedonicum (Corynebacterium), 1032, 1110, 1118
Septoria lycopersici, on tomato, spray control, factors influencing, 9
oudemansii, distribution, 745
Kentucky bluegrass infection by, 745
rubi, leaf spots of blackberry and dewberry due to, 1002
strawberry leaf spot role questioned, 986
Serum, bovine, effect on viruses of plants, 675
SEVERIN, HENRY H. P., 741
SHAN-MING CHEN (see under CHEN)
SHARVELLE, E. G., 1118
SHAW, K. J., (469)
SHAW, LUTHER, (1111), (1116)
SHAY, J. R., 11, (19), (1212)
SHEAR, C. L., (1115)
SHERF, ARDEN F., 330
Shot hole disease, plum-, noninfectious heritable disorder of Beaty var., 1101
stone fruit Coryneum, 506
SISSELMAN, CHARLOTTE B., (314)
SLAGG, CHARLES M., 390
SMITH, A. L., 150
SMITH, CLAYTON O., 82, 1101
SMITH, OLIVER F., 525, 1081
SMITH, RALPH E., 258
SMITH, T. E., 11, 469, 1076
Smuts, barley loose-, 194
seedling-infecting, 324
cereal-, grass susceptibility to, 910
corn-, 749, 943
grass stripe-, 7, 707, 1045, 1116
millet-, 1122
oats-, 1112
oats covered-, 14, 332, 1064
oats loose-, 14, 1064
onion-, 9
wheat-, 258
SNIESZKO, S. F., 1032, (1110), 1118
Snowmold, of grasses and cereals, 1157
Sodium dinitro-o-cresylate, as eradicant spray for Sclerotinia and Coryneum, 515
Sodium nitrite, for damping-off, 1110
for root knot, 1110, 1111
Sodium pentachloro-phenate, as eradicant spray for Sclerotinia and Coryneum, 515
Sodium tetrachloro-phenate, as eradicant spray for Sclerotinia and Coryneum, 515
Soft rot, potato bacterial, charcoal, rot association, 1120
Soil amendments, effect on Fusarium-root knot-attacked cotton yields, 10
Soil treatments, for damping-off, 1110
for root knot, 1110, 1111
fumigants compared, 1118
Soil-borne diseases, Alternaria zinniae, 379
Bacterium tabacum, 1109
pea, control, 1098
Soils, fumigants, adsorption, 12
chloropicrin for root knot, 1166
fungicides, for Fusarium oxysporum cubense, 398
moisture, Lima bean seed treatment vs., 3
Phymatotrichum sclerotia in, inhibition by S autoclaved with soil, 1205
root knot in greenhouse-, control by chloropicrin, 6
sterilization by organic solutions, wetting agent effects, assay, 12
solanacearum (Bacterium), 11, 1076
(Phytomonas), 14
Solanum virus-2, reactions of resistant tobaccos to, 565
Sooty blotch, pear-, 1117
Sorghums, Macrophomina dry rot, 1113
zonate leaf spot (new), 220
Sorghum spp., Gloeocercospora zonate leaf spot (new), 222
halepense, Titaeospora andropogonis on, 220
Sorosporium syntherismae, cross inoculations, 572
interspecific hybridization, 571
life cycle and nuclear condition, 571
SORRELL, M. B., 12, (1114)
SOUTHAM, CHESTER M., 517
Soy bean, seed treatments, effects on emergence, stand and yield, 1113
Specificity, vector-, for virus varieties, 17
Spergon, corn seed treatment by, 602
in pre-harvest cherry sprays, 683
seed protectant value, 9
sweet potato scurf control by, 410
Sphaceloma spp., English ivy leaf scab due to, 1109
hosts in La., 1
**n. sp.**, on Magnolia grandiflora, 6
violae, new records and hosts, 168
Sphacelotheca panici-miliacei, cross inoculations, 572
interspecific hybridization, 571
life cycle and nuclear condition, 571
Sphaeropsis malorum, on apple, Fermate control, 1116
on soft pines, significance, 828
Sphaerotheca humili var. fuliginea, on delphineums, distribution and rapid identification, 832
pannosa, water-soluble fungicide for, 1097
Sphaerulina **rubi** n. sp., raspberry "Septoria leaf spot" due to, 997
Spinach, preemergence damping-off, measure of, seedling germination rate/fungus growth rate as, 7
seed protectants for, 14
Spores, fungus-, on air-exposed slides, identification, 75
eradicant action of fungicides for, on living leaves, 1190
germination method, for Agaricus, 530
viability preserved by quick freezing and storage, 612
Sporobolus spp., Puccinia stakmanii (exp.) on, 387

Spray equipment, new types, efficiency, 1114
Spray injury, copper-, on grape, 13
sulphur-, on apple, 2
Sprays, coverage factor, determination technique, 1114
spreader-stickers, for cherry pre-harvest sprays, 683
Spreaders, in organic fungicides for cherry, 683
STAKMAN, E. C., 12, 424, 884, 943, 1118
STARK, FRANK L., JR., 12, 1118
STARR, G. K., 257
STARR, MORTIMER P., 314
STEINMETZ, F. H., 12, (45)
Stereum purpureum, cordwood decay by, calorific and chemical study, 46
Sterilization, soil-, by organic solutions, assay of wetting agent effects, 12
STEVENSON, JOHN A., (101), 635
stewartii (Phytomonas), 407
Stigmatomycosis, bean Nematospora, 1209
STODDARD, D. L., (162)
STODDARD, ERNEST M., 13, 20, 1119, 1190
STOLLER, B. B., 13
Stone fruits, Armillaria root rot, in eastern U. S. A., 813
STRUBLE, BEN F., 528
Sugar beet, Corticium leaf blight, 6
curly top, on tobacco, as influenced by inoculation site, 10, 586
leaf area determination, technique, 1071
preemergence damping-off, measure of, seedling germination rate/fungus growth rate as, 7
Uromyces betae on, temp. relations and distribution, 10
water-mold seedling disease, temp. and pH relations, 7
Sugar cane, Colletotrichum falcatum on, perfect stage, 2
internal breakdown, mosaic-associated, 3
mosaic, re-infection of recovered plants, 713
virus disappearance from recovered plants, 713
SUIT, R. F., 13
SULLIVAN, J. T., 401
Sulphur, effects on white clover composition by rust and, 401
fungicidal (see under Fungicides)
Phymatotrichum sclerotia inhibited in soil autoclaved with, 1205
Sunflower, crown gall on, sterility of secondary tumors, 85
Sunscald, effect on potato soft rot in transit, 1117
tomato fruit-, effect on leaf blight spray control, 9
Surveys, cereal diseases (seed-borne) in Manitoba, 1111
pea diseases in Wis., 15
Susceptibility, grasses (forage), to smuts of cereals, 910
Suture spot, peach fruit disease (new), cause unknown, 167
Sweet clover, white-, Ditylenchus dipsaci strain on white clover, alfalfa and, 525
Sweet potato, Fusarium wilt, on cotton and tobacco vs., 469
crop rotations for, 483
distribution, 469
scurf, fungicidal control, 410
SWINGLE, R. U., 1196
Symptoms, acute vs. chronic, in virus diseases, 8
Synergism, between toxicants, significance, 18
syringae (Phytomonas), 82
tabacum (Bacterium), 3, 1109, 1120
Taphrina deformans, on peach, early records in U. S. A., 259
TAPKE, V. F., 194, 324
**taraxaci** (Xanthomonas), n. sp., 961
Taraxacum kok-saghyz, bacterial leaf spot (new), 959
Tattoo-netted corky bark, citrus-, virus (?) origin, 862
Taxonomy, phytopathogenic bacterial, culture medium aid to, 314
TAYLOR, A. L., 1166
TAYLOR, CARLTON F., 14, 1119
TAYLOR, J. W., (240)
Technique, abnormal tissues in living pines, detection by dyes, 20
anaerobic collection of plant fluids, 129
bacterial identification by bacteriophage, 1119
chloroplast isolation, 766
chromoprotein extraction from plastids, 768
culture media, agar substitute, corn-meal and starch mush as, 617
for Phytomonas sepedonica, 406
phytopathogenic bacterial growth in synthetic, 314
for potato ring rot bacteria, 1118
cultures, growth measurement of fungus colonies, reflector scale for, 619
maintenance of bacteria and fungi in, 330
single-spore, for Agaricus, 530
distribution (local) of Ribes, measuring, 4
ergot epidemic production, 1116
fungicides, assay, of Bordeaux, 633
correlation of greenhouse and laboratory methods, 15
of effectiveness, 5
of eradicant action, 1194
laboratory-greenhouse method, 19
slide-germination method for protectants, 627
coverage, determination technique, 1114
field testing on apple, 13
particle size determination, 8
retention and protective value determination, 899
fungus growth measurement, reflector scale for, 619
identification of: fungus spores from air-exposed slides, 75
Sphaerotheca humuli var. fuliginea, 832
inoculation, of corn ear and stalk rots, 16
of Typhula, 1158
internal therapy, for vascular diseases, 16

leaf area determination, on potato, 922
on sugar beet, 1071
plot-, for disease control tests on fine turf, 528
sap (plant) collection method, 129
spray equipment, efficiency of new-type, 1114
Verticillium-free clones, establishment, 3
virulence comparison of strains of Alternaria on tomato, 698
virus separation by contact-period effects in grafts, 818
virus symptoms, localization and intensification, 18
virus-protein determination, colorimetric, 8
wetting agent effects on organic soil-sterilizing agents, assay, 12
Terminalia arjuna, fungus (?) leaf spot of, 404
Terminology, fungicidal, definitions, 624
Tetrachloro-para-benzoquinone (see Spergon)
Tetra-methyl-thiuram-disulphide (see Jap-Beetle Spray; Thiosan
THATCHER, F. S., 14
Thea sinensis, Sphaceloma on, 1
Thelephora sp., cordwood decay by, chemical and calorific study, 46
Therapy, internal chemical, for vascular diseases, 16
Thiamin, effect on rust fungi, 724
Thiosan, corn seed treatment by, 602
seed-protectant value, 9
sweet potato scurf control by, 410
as vegetable seed protectant, 14
THOMAS, HAROLD E., (933)
THOMAS, H. EARL, (933)
THOMAS, H. REX, 114, 729, (797), (1176)
THOMAS, JOHN E., 1119
THOMAS, R. C., 1119
THOMAS, W. D., JR., 144
Thrips, as vector of pineapple yellow spot, wind role, 410
Thuja plicata, heartwood extract, effect on wood-destroying fungi, 517
orientalis, Cercospora blight, 1117
THURSTON, H. W., 56
TILFORD, P. E., (1196)
Tilletia levis, on wheat, photoperiodism effects on, 240
tritici, dwarf-bunt race, chlamydospore germination, significance, 732
on wheat, photoperiodism effects, 240
Timber (see Wood)
Tip blight, pine Diplodia, 1018
Tip burn, guttation-fluid role, 1110
Titaeospora andropogonis, on Johnson grass, 220
Tobacco, anthracnose in plant beds, 15
burley, plantago virus outbreak in, 210
Cercospora leaf spot, environal relations, 355
resistance to, real vs. apparent, 358
curly top, immunity acquirement, mechanism, 598
inoculation site effects, 10, 586
downy mildew, distribution and taxonomy, 101
etch, hosts (exp.), 1114
on Physalis subglabrata, 1114
Fusarium wilt, on cotton and sweet potato vs., 469
crop rotations for, 483
distribution, 469
Granville wilt, control by urea in soil, 11
pattern of occurrence in successive plantings, 1076
leaf-spot bacteria, multiplication on roots of other spp., 3, 1120
mosaic, klendusity to, in Chilean tomato and hybrids, 19, 691
N and N′ factors in relation to, 14
resistant genotypes, 300
tomato derivatives escaping, 19
virus, cyanide effects on synthesis, 77
origin and relationships, 219
sensitivity to inactivators, 675
virus-1, acute and chronic phases and factors influencing, 1052
virus-protein, colorimetric determination, 8
pathogenicity of miscellaneous bacteria vs. Bacterium tabacum to, 1109
ring spot, reactions of resistant tobaccos to, 565
virus, cyanide effects on synthesis, 77
sensitivity to inactivators, 675
Turkish, virus passage from cion to stock in, contact period effects on "yellow" vs. "mosaic" type viruses, 818
viruses, acute vs. chronic symptoms by, 8
virus-strain reactions of resistant, 551
yellow mosaic, acute and chronic phases and factors influencing, 1045
tolaasi (Phytomonas), 1093
Tomato, Alternaria early blight, "physiological" maturity vs., 1
seed treatments for, 797
in seedlings, culture practices influencing, 1176
sporulating non-chromogenic strain from, 729
virulence of strains, comparison technique, 698
anthracnose, control, by Fermate vs. copper fungicides, 1120
bacterial wilt, nitrogen utilization by pathogen, 14
broom-rape parasitizing, 257
curly top via tobacco cions on, reactions to, 597
Fusarium wilt, pathogenesis, toxin role, 4, 126, 1111
toxic effects of extracts of mild vs. virulent strains, 1004
toxins in plants with, 4
virulence variations and host reactions, 175
leaf blights, spray control, factors influencing, 9
plantago virus (exp.) in, 216
"red ring" of stems, insect-induced, 613
root knot on, treatment for greenhouse control, 6

seed protectants for, 14
tobacco mosaic klendusity (heritable) in Chilean tomato and hybrids with, 691
vascular wilt, Fusarium retusum (new) causing, 956
Toxicants, in crop residues, role of microorganisms in, 1111
synergism and antagonism between, significance, 18
"Toxins," in tomato Fusarium wilt, 4, 126, 1004, 1111
virus vs. fungus, 20
Trametes spp., as influenced by heartwood extracts of western red cedar, 518
serialis, black cherry rot due to, general study, 984
suaveolens, sporophores, environal effects on morphology, 328
Tranzschelia pruni-spinosae, on peach, control by Fermate, 962
Trees, forest-, diseases, book review, 338
seeds, book review, 338
fruit-, Armillaria root rot in eastern U. S. A., 812
fungicides on, field testing technique, 13
nursery-, root rot and control in deciduous, 656
Trichothecium **arrhenopum** n. sp., Pythium oospores parasitized by, 232
Trifolium repens, Ditylenchus dipsaci strain on alfalfa, sweet clover and, 525
as influenced by rust and sulphur, 401
Trioxymethane, fungicidal action of vapor, 445
Triticum timopheevi, stem rust on, 335
TROPICAL PLANT RESEARCH FOUNDATION, dissolution, announcement, 1107
Truffle disease, of mushrooms, control by ammonia, 13
Trypsin, effect on viruses of plants, 675
Tulip tree, root rot in nursery, causes and control, 656
tumefaciens (Bacterium), 8
(Phytomonas), 85, 1119
Tumors, invading plant-, factors influencing, 8
malignancy in animal- vs. plant-, 98
plant-, chemical influences resembling, 1119
Turf, fine-, disease control tests on, plot technique, 528
Typhula sp., snowmold of pasture grasses due to, hosts and general study, 1157

Ultra-viruses (see Viruses)
Uncinula necator, on grape, copper fungicides for, control vs. spray injury, 13
UNION OF AMERICAN BIOLOGICAL SOCIETIES, committee report on, 419
Urea, tobacco Granville wilt control by, 11
Urocystis spp., from cereals, grass (forage) susceptibility to, 910
agropyri, physiologic races, 919
tritici, origin, 920
physiologic races vs. wheat vars., 169
Uromyces betae, on beet, temp. relations and distribution, 10
caryophylinus, on carnation, eradicant action of fungicides on leaves, 1191
phaseoli, fungicidal action on, factors in, 1147
trifolii, effects of sulphur and, on white clover composition, 401
Ustilago spp., of barley loose smuts, occurrence, identification and species validity, 194
avenae, on oats, seed treatments for, 1064
physiologic races, exptl., 14
crameri, cytology, life history and host relations, 1122
hordei, on barley, seed treatments for, 1064
levis, on oats, seed treatments for, 1064
physiologic races, exptl., 14
medians, taxonomic status, 206
nigra, amplified description, 206
on barley, physiologic races, frequency and distribution, 324
distribution, 206
striaeformis, chlamydospore germination, temp. effects, 1055
isolation and culture from bluegrass, 1116
on Kentucky bluegrass, host development vs. temp., 1055
f. agrostidis, chlamydospore germination, 707
culture, 7, 707
f. poae-pratensis, chlamydospore germination, temp. effects, 7
zeae, chlamydospores, heritable abnormality in, 749
promycelium lysis inherited, 751
white mutant character, inheritance, 943
UTTER, L. GORDON, 14

VALLEAU, W. D., (3), 14, 210, 1120
Variegations (plant) (see also Viruses)
citrus infectious, crinkle leaf association, 860
cytology and histology, 641
mosaic-like, in snap beans, 779
origin and relation to virus infections, 637
sexual inheritance, 650
symptoms, 640
VAUGHAN, EDWARD K., 14, (797)
Vectors, insect- (see Insects as vectors)
Vegetables, boron deficiency in, 940
Pythium myriotylum soft rot, 261
seed protectants for, new organics, 14
seed treatments for, report of Coordination of Research Committee, 423
Venturia inaequalis, on apple, control, eradicant action of fungicides on leaves, 1192
by eradicant vs. protective sprays, 6
by eradicant sprays, cationic phenyl Hg compounds as, 1114
by Fermate, 5
fungicidal, particle size vs., 533
spray effects on tree growth and yield, 56

sulphur coverage vs., 1119
water-soluble fungicide for, 1097
conidia, preservation of viability by quick freezing and storage, 612
mutant characters, genetic studies, 11, 19
pathogenicity, inheritance, 19
Verticillium spp., clones free of, establishment, 3
internal therapy against, 16
toxins of, virus compared, 20
Viability, of fungi and spores, preservation by quick freezing and storage, 612
Violet, Sphaceloma scab, new records and hosts, 168
VIRGIN, W. J., 743
Virulence, of Alternaria strains on tomato, comparison technique, 698
Viruses (plant—special and general data; see also Variegations)
behavior in resistant hosts, 311
classification and nomenclature, committee report, 424
disease symptoms, acute vs. chronic, 8
genetics of expression, 16
masking by temp. in cherry yellows, 1213
techniques for localizing and intensifying, 18
evolution, from chondriome (?), 20
from mitochondria and derivatives, chemical evidence, 766
cytological and genetical evidence, 637
immunity acquirement in tobacco, mechanism, 598
nature and biological activity, 950
pathological growth and symptoms due to, chemical influences resembling, 1119
recovery from infection in sugar cane mosaic, 713
sensitivity to inactivators, 674
separation by contact-period effects in grafts, 818
severity of attack, inoculation site effects, 10, 586
toxins of fungi vs., 20
vector specificity for vars. of potato yellow dwarf, 17, 1110
"yellow" vs. "mosaic" types, passage from cion to stock in Turkish tobacco, contact period effects, 818
Viruses (by plant hosts; for details see under hosts and specific diseases)
aster, yellows, 2, 741
potato infection, 16
bean, mosaic, 778
mosaic (new), 1215
new disease (?), 743
virus-4, 16
carnation, mosaic, 823
cherry, 6
rasp leaf, 620
yellows, 6, 449, 1213
citrus, bark disorders (?), 860
convex gum, 394
psorosis, 865
varieties of, 837
cranberry, false blossom, 19
cucumber, mosaic, 675, 818
virus-1, 565
elm, mosaic, 1196
fig, mosaic (?), 719
peach, yellow-red, 18, 20
pineapple, yellow spot, 408
plantago, mosaic, 564
in tobacco, 210
potato, 19
purple-top wilt, aster yellows causing, 16
ring spot, 675
X-mosaic, 1116, 1216
yellow dwarf, 363, 1110
on Nicotiana rustica, 1121
raspberry, decline, 156
sugar beet, curly top, 10, 586, 818
sugar cane, mosaic, 3, 713
tobacco, 8, 551
curly top on, 10, 586
etch, hosts (exp.), 1114
mosaic, 8, 14, 77, 219, 300, 675, 691
on tomato, 19
ring spot, 77, 675
Nicotiana virus-1, 1045
and mutants, 551
yellow mosaic, 1045
tomato, ring spot, 818
tobacco mosaic on, 19
Vitamins, effect on rust fungi, 724

WALKER, E. A., 15
WALKER, J. C., 778
WALLACE, H. A. H., 4, (1064)
WALLACE, J. M., (865)
WALLACE, PHILIP P., (1121)
Walnut, black- (see Juglans nigra)
WANG, C. S., 1122
War Committee, of American Phytopahtological Society, reports, 339, 424, 747
Washingtonia filifera, bacterial leaf disease (new), 1201
**washingtoniae** (Phytomonas), n. sp., 1203
Water-core, in rutabaga, control by borax in soil vs. spray, 8
WATERMAN, ALMA M., 828, 1018
Watermelon, preemergence damping-off, measure of, seedling germination rate/fungus growth rate as, 7
WATSON, R. D., 1120
WEAVER, L. O., (5), 15, (612)
WEBER, GEORGE F., 615
Weeds, tobacco leaf-spot bacteria on roots of, 1120
WEIMER, J. L., 249, 319
WEISS, JAMES E., (314)
WELLMAN, FREDERICK L., 175, 617, 698, 956, 1004
WELLMAN, R. H., 15, (19)
WERNHAM, C. C., 1157
Wetting agents, effect on organic soil-sterilizing agents, assay, 12
Wheat, Alternaria on seeds, storage temp. effects, 333
"black point," distribution, etiology and control, 1112
bunt, copper fungicides for, early use in U. S. A., 258

photoperiodism effects on, 240
seed treatment by Spergon, 332
dwarf bunt, chlamydospore germination, 732
Helminthosporium sativum on seed, in Canada, 4
kernel smudge, varietal reactions to, 4
loose smut, physiologic races, 169
powdery mildew, on grass spp., 5
Pythium ostracoides (new) on roots, 275
seed-borne diseases, seed treatments for, 1064
smut, copper fungicides for, early use in U. S. A., 258
stem rust, browning reaction, host vars. and rust traces vs., 484
population trends (U. S. A., 1930–41), 884
reactions as influenced by vitamins and growth substances, 724
regional spread from barberries (1942), wind role, 12
resistance mechanism, 14
testing for resistance, technique, 897
on Triticum timopheevi, 335
WHITE, PHILIP R., (85)
White mosaic, reactions of resistant tobaccos to, 563
WILCOX, MARGUERITE S., (986)
Wildfire, tobacco bacterial, 3, 1109, 1120
WILHELM, STEPHEN, 806
WILSON, E. E., 497, 506, 962, 1120
WILSON, J. D., 1120
Wilting, effect on susceptibility to tomato early blight, 1179
Wilts, banana Fusarium, 835
cotton Fusarium, 10
flax-, 1113
potato purple-top, aster-yellows virus causing, 16
tobacco Granville bacterial-, 11, 1076
tomato bacterial-, 14
tomato Fusarium, 4, 126, 175, 1004, 1111
tomato Fusarium (new), 956
vascular, internal chemical therapy, 16
Verticillium, 3
Wind (see under Air)
Winter injury, of plane tree (London), infections following, 963
WOLFENBARGER, D. O., (76)
Wood, decayed, calorific and chemical study, 45
fungi destroying, as influenced by heartwood extracts of western red cedar, 517
living, abnormal tissues in, detection by dyes, 20
preservative, new adaptation, 17
Woods, M. (see Woods, Mark W.)
WOODS, MARK W., 20, 77, 637, (766)
WORTHLEY, H. N., 56
WRIGHT, CHARLES M., (12)
WRIGHT, ERNEST, (390)

Xanthomonas spp., growth in synthetic medium, as taxonomic aid, 315
**taraxaci** n. sp., dandelion (Russian) leaf spot due to, 961
X-disease, peach (see Yellow-red virosis)
X-virus, potato, foliar mottle and necrosis of Chippewa var. associated with strain of, 1116, 1216

YARWOOD, C. E., 1146
Yeast spot, bean Nematospora, 1209
Yellow dwarf, potato, on Nicotiana rustica, local lesion factors, 1121
vector specificities for vars. of, 17, 1110
virus, clover leaf hopper transmission relations, 363
Yellow mosaic, tobacco, acute and chronic phases and factors influencing, 1045
Yellow-mosaic virus, type-B, reactions of resistant tobaccos to, 564
type-C, reactions of resistant tobaccos to, 565
Yellow-red virosis, peach, symptoms, techniques for localizing and intensifying, 18
virus, fungus toxins compared, 20
Yellow spot, pineapple, plant spacing vs. incidence, 408
wind-blown vector, 410
Yellows, aster, on Ambrosia artemisiifolia, 16
virus, annual-phlox infection via leaf hoppers, 741
potato infection, 16
properties, 2
transmission by Macrosteles divisus, 16, 741
cabbage Fusarium, 15
cherry, new virus, 449
insect transmission, 466
strains on Montmorency var., 6
symptom masking by temp., 1213

YORK, HARLAN H., 20
YOUNG, GEORGE Y., 656
YOUNG, H. C., JR., 16, 1121
YOUNKIN, S. G., 16, 1121

ZAUMEYER, W. J., 16
ZEEUW, D. J. DE, 530
ZELLER, S. M., 136, 156
ZENTMYER, GEORGE A., JR., 16, (20), 1121
Zinnia, Alternaria disease, general study, 372
Zizania sp., Claviceps on, 12

## ERRATA, VOLUME XXXIII

Page 2, line 11, *read* r. p. s. *for* r. p. m.
Page 204, line 5, *read* (Fig. 3, D) *for* (Fig. 4, D)
Page 220, line 6 from bottom of page, *read Cercospora for Cerospora*
Page 225, line 1, *read Gloeocercospora for Gloeocerspora*
Page 308, line 6, *read* seeding *for* seedling
Page 328, line 12, *read* 50 cc. *for* 500 cc.
Page 430. In date line beneath title, *read* July 29 *for* July 28
Page 552, line of continuation of footnote 4 of preceding page *read* dark green *for* green dark
Page 567, literature citation 2 *read* Dalldorf *for* Daldorf
Page 624, delete footnote [2] from title
Page 628, lines 25 and 49, *read* McCallan *for* McAllan
Page 882, line 8, *read* yellows *for* yellow
Page 952, paragraph 3, line 6, *read* $H_2S$ *for* H.S.

# PHYTOPATHOLOGY

VOLUME XXXIII

1943

# ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE THIRTY-FOURTH ANNUAL MEETING OF THE SOCIETY, NEW YORK, N. Y., DECEMBER 28 TO 31, 1942[1]

*Plants Attacked by Species of Sphaceloma in Louisiana.* ARRUDA, S. C., AND C. W. EDGERTON. Following observations made by A. A. Bitancourt of Brazil during a visit to Louisiana in 1941, attention was directed to the hosts and species of *Sphaceloma* in the state. *Sphacelomas* have been collected and cultured from *Camellia japonica, Camellia sansanqua, Thea sinensis, Punica granatum, Bignonia capriolata, Catalpa bignoniodes,* and *Sambucus canadensis.* Some of the cultures were similar but some were distinct. One culture of the fungus from Camellia, apparently the cause of a white scab that has been observed in Louisiana for many years, seems unlike any described form. Ascomata of the Elsinoë stage did not occur during the summer months but some were beginning to be found during October.

*Investigations in the Relationship Between Alternaria Blight and "Physiological" Maturity in the Tomato Plant.* BARRATT, R. W., AND M. C. RICHARDS. Some of the factors affecting "physiological" maturity in the tomato were investigated in the field and greenhouse. The following factors were considered: Fruit to leaf ratio; planting date; supply of nitrogen; phosphorus and potassium as fertilizers; spraying with Bordeaux mixture; and total yields. Yield data were obtained on each of 10 plants from 50 varieties. The incidence of disease (*Alternaria solani*) was recorded weekly. Tests were made in the greenhouse on an early and a late variety to determine the effect of fruit load and nitrogenous fertilizers on disease incidence. It was found that those factors delaying "physiological" maturity—defruiting and late planting—retarded defoliation by Alternaria. Those factors that accelerated "physiological" maturity—early planting and partial deleafing—hastened defoliation. In the species *L. esculentum, L. pimpinellifolium, L. hirsutum,* and *L. peruvianum,* and in 45 commercial types, defoliation increased as the fruit-to-leaf ratio increased, and as the peak yield occurred earlier in the season. In 1941, certain lines were chosen from breeding material for disease resistance to Alternaria blight; when these selections were grown in 1942, it was found that they were not disease resistant but merely later maturing varieties.

*Boron in Relation to Internal Bark Necrosis of Apple.* BERG, ANTHONY, AND GENEVIEVE CLULO. The results of 3 seasons' experiments in the orchard have shown no direct relationship between boron content of apple tissues and the occurrence of internal bark necrosis. The boron content of leaves, bark, wood, and fruits from diseased trees was as high as that from disease-free trees. Heavy applications of boron were made in the spring to the soil surrounding diseased trees. Analyses of the tissues of these trees during the same growing season showed that conideriable quantities of boron had been accumulated by all tissues. Yet, at the end of the growing season, the disease developed on the new growth of some of these trees. A 2-year study of the tissues of 14 varieties of apples from a given orchard showed no significant difference in the boron content of susceptible and immune varieties. Trees grown in sand culture in the greenhouse for 4 successive seasons without boron did not develop the disease, while potted trees grown in the same greenhouse in soil taken from affected orchards developed the disease at the end of the first growing season.

*Scab of Mango Caused by Elsinoë.* BITANCOURT, A. A. AND ANNA E. JENKINS. Diseases caused by *Elsinoë* are now known to affect more than ten important tropical fruits. Among them is the mango (*Mangifera indica* L.), on which is produced numerous small leaf, stem, and fruit spots. Severe attacks under nursery conditions at Santiago de las Vegas, Cuba, resulted in crinkling, as well as shedding of young leaves. On this young growth, spots are pale to brown and are covered with a delicate buff down, the conidial stage of the pathogen. This is morphologically similar to *E. fawcetti.* The somewhat larger scabs on older mango leaves are gray above, surrounded by a dark marginal line. On them are produced the small, nearly black ascomata of the *Elsinoë.* At São Paulo, Brazil, spots are chiefly epiphyllous, circular, to elongate or irregular, and gray at the center, with a dark periphery. They follow the midrib or are disposed more or less close to it. At Manaos, in the state of Amazonas, Brazil, and also in Puerto Rico, Florida and Canal Zone symptoms resemble those just described for both Cuba and São Paulo. The *Elsinoë* is described as a new species. Isolations consist of a single conidium culture from a Cuban specimen and a tissue culture from São Paulo.

[1] Meeting was canceled on action of A. P. S. Council.

*Some Properties of Aster-Yellows Virus.* BLACK, L. M. Properties of aster-yellows virus were studied in juice of viruliferous insects (*Macrosteles divisus* (Uhl.)) after dilution to $10^{-1.5}$ in 0.85 per cent NaCl solution brought to pH 7.0 by addition of $K_2HPO_4$. Active virus was detected after 24, but not 48, hours at 0° C.; after 2, but not 3, hours at 25° C.; after 10 minutes at 35° C.; but after 10 minutes at 40° C. no virus could be detected in one experiment and only a trace in a second. In preliminary experiments, active virus remained after treatment, for less than 5 minutes, at pH 5.0 or 9.0. Virus passed with difficulty through Berkefeld N and V filters that retained *Serratia marcescens* Bizio but did not prevent passage of a bacterium which was apparently a normal contaminant of the preparations. Solutions that were spun for 1 minute at a top speed of 500 r.p.m. (plus 8 minutes' acceleration and 8 minutes' deceleration) yielded sediments which, when resuspended in the original volume of saline, were highly active. The supernatant fluids were inactive. The results from filtration and ultracentrifugation suggest that activity is associated with a particle that is of large size relative to plant viruses heretofore studied.

*Influence of Boron Status on Cold-storage Behavior of Apple Fruits.* BURRELL, A. B. Small lots of McIntosh or Fameuse apples in contrasting boron status have been stored 6 different years. The first 4 years, no boron treatments had been made; the comparison was between crops of trees where boron deficiency symptoms were present in part of the fruits and crops free from such symptoms. In 3 of the years, no difference in storage behavior was noted, while in 1 there was more flesh browning during storage in fruits from boron-deficient trees. The last 2 years, apples from trees receiving boron were compared with those from nontreated trees very slightly deficient in boron. In one test with Fameuse, storage breakdown near the core was worse in fruits from nontreated trees. With McIntosh, application of about 5 times the usual amount of boron appeared to have slightly increased storage breakdown. Otherwise, treated and nontreated behaved similarly. Never was there a difference in fungous decay. Six rather heavy annual applications of boron appeared, in 1942, to have advanced maturity and increased drop of McIntosh, where nontreated trees had adequate boron. If, as some reports indicate, excessive boron should reduce storage life of apples, perhaps very light annual soil applications would be preferable to the usual triennial ones.

*A Six-year Comparison of Lime-Sulphur and Flotation Sulphur as to Yield and Growth of Young McIntosh Apple Trees.* BURRELL, A. B. This experiment was conducted in the relatively cool, dry climate of the Champlain Valley of New York, which is not especially conducive to spray injury. Trees, 10 years old at the outset in 1937, moderate in vigor and just commencing to bear, were given 4 treatments: (A) lime-sulphur 2–100 all season; (B) ferrox flotation sulphur all season, (C) lime-sulphur early (usually 2 applications) followed by flotation sulphur (usually 3 applications), and (D) flotation sulphur early followed by lime-sulphur. Lead arsenate, usually 3 lb. per 100 gal., was included in all applications, and in some, an equal amount of hydrated spray lime. Each treatment was applied to 16 individual-tree replicates arranged in four, $4 \times 4$ Latin squares. The treatment for a given tree was the same throughout the 6-year period. Compared with treatment B (flotation sulphur all season), treatment A (lime-sulphur all season) reduced yield by about one third; treatment D (lime-sulphur late) reduced it slightly less; treatment C (lime-sulphur early) perhaps still less. The spread between the respective treatments was greatest in 1942. The percentage of blemish-free fruits differed little among treatments for the period as a whole. Terminal elongation and trunk enlargement were uninfluenced.

*The Perfect Stage of Colletotrichum falcatum.* CARVAJAL, F., AND C. W. EDGERTON. During 1942, a fungus belonging to the genus *Physalospora* was found very abundant on old dead leaves and leaf sheaths of sugar cane in Louisiana. The perithecia are inconspicuous, entirely embedded in the leaf tissue. In inoculation experiments involving cultures obtained from single ascospores, typical red rot lesions with conidia characteristic of *Colletotrichum falcatum* Went were obtained. The perithecia were produced also by inoculating sterilized leaves in moist chamber with pure cultures of *C. falcatum* and by placing leaves inoculated in the field in moist chambers. The perithecia fit closely the description of *Physalospora tucumanensis* Speg. Material of *P. tucumanensis* kindly sent by Juan C. Lindquist of the Institute de Botanica "Spegazzini," La Plata, and labeled "No. 418, separado del tipo," though dated after the original description of the fungus, agreed reasonably well with the Louisiana material, except that the perithecia seemed to be slightly larger. Tentatively, the Louisiana fungus is being considered as *Physalospora tucumanensis* Speg.

*Certain Organic Materials in Relation to Copper Compounds for the Control of Downy Mildew of Lima Beans.* CUNNINGHAM, H. S. The 1942 season witnessed the most

widespread and severe infestation of downy mildew of Lima beans that Long Island growers have experienced for several years. Under such conditions certain organic materials now on the market showed little promise of becoming reliable substitutes for copper for the control of this disease. Using data based on the number of clean pods per acre the organics were little better than no treatment and far inferior to most copper compounds. No treatment used was so effective as Bordeaux mixture.

*The Role of Soil Moisture in Relation to Chemical Seed Treatment of Lima Beans.* CUNNINGHAM, H. S. Under Long Island conditions and with the Fordhook variety, chemical seed treatment has failed to hasten the emergence of Lima beans, while at best the results have been erratic in so far as improvement of stand is concerned. Experiments have shown that soil moisture at planting time may be the most important factor assuring that quick emergence so necessary in combating injury caused by wireworm and seed corn maggot. The addition of as little as 200 gal. of water per acre at planting time has hastened emergence and the final stand has compared favorably with any of the chemical treatments used at present.

*Multiplication of Tobacco Leaf-spot Bacteria on Roots of Other Species.* DIACHUM, STEPHEN, W. D. VALLEAU, AND E. M. JOHNSON. Roots of seedlings of several unrelated species of plants were dipped in a 1–1000 dilution of a broth culture of *Bacterium tabacum*, and the plants were potted in sterile soil. Ten plants of each species were removed from the soil immediately. The roots were washed in running tap water, crushed in water, and poured on water-soaked tobacco leaves. Very few, if any, wildfire spots developed on the leaves. The roots of 10 plants of each species were then tested in 24 hours, and another set 96 hours after they had been dipped in the bacterial suspension. In each case large numbers of wildfire spots developed on the leaves, showing that the tobacco leaf-spot bacteria multiply rapidly on the roots of plants, including wheat, oats, barley, rye, cowpeas, soybeans, castor beans, alfalfa, vetch, and crimson clover.

*A Method of Establishing Verticillium-free Clones of Perennial Plants.* DIMOCK, A. W. Based on the assumption that invasion of the shoots of verticillium-infected plants occurs only by growth of hyphae upward through vascular tissues, the following method of obtaining verticillium-free propagating material from infected chrysanthemum stock plants was devised. Shoots 4 to 6 inches long were taken from the stock plants. Slices were cut from the base of each shoot and plated out on potato-dextrose agar. The Petri plates and the shoots were labelled similarly and the latter placed in cold storage. Ten to 20 days later the plates were examined and shoots yielding Verticillium were discarded. Cuttings were made from the remaining, presumably healthy, shoots, rooted in sterilized sand and grown in sterilized soil and containers. Further cuttings were taken in the usual manner (without culturing) from the resulting plants as rapidly as new shoot growth developed. Verticillium-free clones of propagating stock, thus obtained, could be maintained indefinitely if grown continuously in verticillium-free substrates. The method is at present being used very successfully by a large producer of chrysanthemum cuttings, and may well be adapted to the production of verticillium-free clones of such plants as phlox, fall monkshood, hardy asters, ornamental shrubs, bush fruits, etc.

*Variation in Single Basidiospore Cultures of Rhizoctonia solani.* EXNER, BEATRICE, AND S. J. P. CHILTON. A total of 395 single basidiospore cultures of *Rhizoctonia solani* isolated from basidial mats formed on Lima bean, Irish potato, and alligator weed stems were compared in culture. As many as 29 distinct cultural types differing in rate of growth, color, size and position of sclerotia, and zonation were found to occur among the isolates from a single basidial mat. These results suggest that some type of segregation occurs in the formation of basidiospores.

*Internal Breakdown of Sugar Cane Associated with Mosaic.* FORBES, I. L., AND JEAN DUFRENOY. A disease of sugar cane characterized by the breaking down and collapse of the central pith cells in rather definite elongate areas in the internodes was observed in the variety C.P. 33/243 in 1941. The necrotic areas were distributed in various internodes from the base to the top of the stalk but usually did not occur in all internodes. Noticing that these areas were in mosaic-infected stalks, many stalks were examined. In 1941, of 22 stalks with mosaic, all showed internal breakdown, while none was present in more than 200 stalks of mosaic-free cane. In 1942, of 64 stalks with mosaic, all showed internal breakdown, while 2 out of 39 of apparently mosaic-free stalks each showed a very small lesion. These small lesions, however, begin to show as soon as do the symptoms of mosaic on the leaves. It is apparent that the internal breakdown is a symptom of mosaic in this variety. Free-hand sections through lesions show that the vacular contents (mostly phenol compounds) leak out into the intercellular spaces. These, as well as the cell walls, stain a deep red with chlorhydric phloroglucin. The material left in the cells flocculates

into a brown sediment and settles to the bottom of the cells. Internal breakdown has been observed in the past in other varieties.

*Measuring the Local Distribution of Ribes.* FRACKER, S. B., AND H. A. BRISCHLE. In a study of the methods of checking field work on the blister-rust-control project at several locations in Idaho, Washington, and California, it was found that *Ribes viscosissimum* and *R. roezli* were distributed as if a "contagious" distribution were superimposed on a random "Poisson" distribution in the proportion of about two to one. The term "mixed" distribution is proposed for this condition. The mean number of ribes per 100 quadrats and the percentage of quadrats occupied proved more useful measures of the type of distribution than did the mean ribes population per acre. Divergence from a random distribution could be successfully and conveniently measured either by dividing the variance by the mean, or by dividing the actual mean ribes per 100 quadrats by the number that would be expected in the case of a strictly random distribution in which the observed percentage of quadrats were occupied. Analysis of the variance between different species or areas can be handled by employing either a Poisson transformation or a probit-logarithmic transformation, both of which are described.

*The Presence of Toxins in Tomato Plants Affected with Fusarial Wilt.* GOTTLIEB, DAVID. Tracheal fluids obtained by the centrifuge method from tomato plants infected with *Fusarium bulbigenum* var. *lycopersici* contain toxins that cause wilting of tomato seedlings. Three-week-old seedlings wilted when placed in tracheal fluids from diseased plants but remained turgid when placed in similar fluids from healthy plants. Dilution experiments indicate that wilting is due to toxins and not to osmotic pressure. The toxin is not the result of wilting as such because seedlings remained turgid when placed in tracheal fluids of plants that had wilted because of insufficient soil moisture. Thus toxins apparently are formed only when the pathogen is present in the vascular system of the host. On the other hand, seedlings wilt, when placed in extracts obtained by hydraulic pressure from healthy, as well as diseased, plants, and this is not due to osmotic pressure of the extracts. (Minnesota Agricultural Experiment Station, University Farm, St. Paul, Minnesota.)

*Life History and Pathogenicity of Glomerularia lonicerae.* GOULD, C. J. *Glomerularia lonicerae* is found abundantly every year on the leaves of species of *Lonicera* in Iowa. Often on diseased tissue there occur 2 spore stages of the pathogen, the glomerularia conidial stage and a basidiospore stage. The basidia arise from binucleate intercellular hyphae, protrude through stomata, and become transversely septate into 4 cells on each of which is produced a basidiospore. At about the same time binucleate hyphae grow into the upper epidermal cells and form intracellular structures. Binucleate conidia may develop subsequently on the lower surface. Isolates from diseased leaf tissue, conidia, single basidiospores and masses of basidiospores produced similar mycelia and conidia in artificial culture, but never basidia. The pathogen invades parenchymatous and vascular elements, but causes no or only slight distortion of the tissues. Infection was readily obtained with basidiospores, but never with conidia. Infection was favored by temperatures of 15 to 21° C., relative humidity near 100 per cent, young leaves and exposure of the lower surfaces. Symptoms developed within 10 to 20 days, depending on environmental conditions. Tests showed that many species and varieties of *Lonicera* are susceptible. One variety has been found that is apparently immune, *L. japonica* T. *halliana* (D.) N.

*Prevalence of Helminthosporium sativum in Wheat and Barley Seed in Canada.* GREANEY, F. J., AND J. E. MACHACEK. Seed surveys and plating tests made with a large number of samples of wheat and barley seed-grain produced in different Provinces of Canada from 1937 to 1941 showed that among the numerous organisms isolated, *Helminthosporium sativum* was the predominating pathogen. The average percentage of seeds infected with this fungus, for 2,723 wheat samples and 1,311 barley samples, was 2.6 per cent and 5.5 per cent, respectively. Internal seed infection with *H. sativum* varied y from year to year, from Province to Province, and even from field to field. Thus, the average percentage of wheat and barley seeds infected by *H. sativum* in samples from the 1937 crop was 5.3 per cent and 9.8 per cent, respectively; while the percentages for 1939 were only 1.1 per cent and 4.9 per cent. Samples of wheat and barley of the 1939, 1940, and 1941 crops from the Maritime Provinces had 3.2 per cent and 21.6 per cent infection, respectively; while the 1939, 1940, and 1941 samples from British Columbia had less than 0.5 per cent infection. In Manitoba, marked differences in prevalence of *H. sativum* occurred in different localities. The most severe seed infections occurred in localities having the highest rainfall during the growing season.

*Varietal Susceptibility to Kernel Smudge in Wheat.* GREANEY, F. J., AND H. A. H. WALLACE. A number of wheat varieties were tested for their susceptibility to kernel

smudge caused by species of *Alternaria* and *Helminthosporium sativum* in trials at several stations in Western Canada from 1935 to 1942. Of the varieties tested, those belonging to *Triticum durum* were more susceptible to kernel smudge than those of *T. vulgare*. The varieties of hard red spring wheat tested, ranked in order of susceptibility to kernel smudge as follows: Apex, Thatcher, Marquis, Red Bobs, Renown, Regent and Garnet. In all tests, Apex and Thatcher were more susceptible than Renown and Regent. The results of extensive plating tests with a large number of grain samples of Apex, Thatcher, Renown, and Regent of the 1939, 1940, and 1941 crops showed that the varieties Apex and Thatcher were more susceptible to internal seed infection by *Alternaria* spp. and *H. sativum* than Renown and Regent. In all samples tested, the percentage of kernels yielding these fungi was considerably higher than the percentage of kernels exhibiting typical external symptoms of smudge. On the other hand, the degree of internal kernel infection was always positively associated with the incidence of kernel smudge in the threshed grain.

*Redistribution of Fungicides on Apple Foliage.* HAMILTON, J. M., G. L. MACK, AND D. H. PALMITER. A new concept in evaluating fungicidal effectiveness is based upon the discovery that redistribution of the toxicant from the initial spray residue is an important factor in disease control. Experiments with sulphur and Fermate indicate that a definite balance between the retained and distributed portions of the original deposit is essential. Under controlled conditions, light rainfalls of 0.1 inch or less caused enough movement of toxicant on a single leaf to protect non-covered areas against the apple scab and cedar-apple rust fungi. In field experiments, the toxicant was transferred from leaf to leaf in sufficient quantity to give disease control with rainfalls varying in amounts from 0.1 to about one inch. Although it was found that 50 to 80 per cent of the initial deposit was removed with 0.1 inch of rain, about 10 per cent of this was transferred to unsprayed foliage. To obtain disease control on such unsprayed foliage, a minimum concentration of 4 to 5 lb. of actual sulphur per 100 gal., irrespective of the nature of the material, is required. (This concentration corresponds to a deposit of 400–500 micrograms of sulphur per 10 sq. cm.) Oil stickers have not been found to interfere with the transference of sulphur.

*Evaluation of Fermate for the Control of Apple Scab and Cedar-apple Rust Fungi.* HAMILTON, J. M., D. H. PALMITER, AND L. O. WEAVER. Three years' data show that Fermate (ferric-dimethyl-dithio-carbamate), formerly IN870 A3, is the most promising substitute for sulphur and copper for the control of apple diseases in New York State. The material is unusually specific for *Gymnosporangium* sp. Fermate can replace copper in the cover sprays. A combination of Fermate and a wettable sulphur has possibilities. Lime increases the toxicity of Fermate. Fermate appears to be of nutritional value to the tree. Fermate may be redistributed on a given leaf and from leaf to leaf sufficiently to protect unprotected foliage. Fermate, 1½–100, is a minimum concentration to meet the redistribution and disease-control requirements. Fermate, sprayed on the lower surface of the leaf, prevents infection from *G. juniperi-virginianiae* (and to a lesser degree, *Venturia inaequalis*) on the upper surface; but, if sprayed on the upper surface, infection occurs on lower surface. Under certain conditions, sufficient toxicant is translocated in an individual growing leaf and into adjacent unsprayed leaves to give protection, but not to new growth. The amount of toxicant present, the hours of moisture before infection periods, and the degree of susceptibility (particularly as related to rainfall) are limiting factors. Jap Beetle Spray, a compound chemically similar to Fermate, gave comparable results.

*Pathogenicity of Races of Erysiphe graminis on Grasses in the Tribe Hordeae.* HARDISON, JOHN R. *Erysiphe graminis* from wheat and barley infected species of the genera *Aegilops, Agropyron, Elymus, Sitanion, Triticum,* and *Agropyron, Elymus* and *Hordeum,* respectively. From a natural infection on *Agropyron repens* one isolation produced infection on barley, *Agropyron repens* and a slight infection on *A. intermedium.* The "mildew-resistant" barley variety, Arlington C.I. 702, proved very susceptible. Another isolation from the same source infected grasses in *Aegilops, Agropyron, Elymus, Hystrix,* and *Sitanion.* A mixed culture from *Elymus dahuricus* infected barley, many wild grasses, and wheat. An isolation from the latter infected species of *Aegilops, Agropyron, Elymus, Hystrix, Sitanion* and *Triticum.* Another isolation from *Elymus dahuricus* infected only species of *Agropyron* and *Elymus.* Thus, mildews from wheat, barley, *Agropyron,* and *Elymus* occur either separately or together as mixtures on certain species of *Agropyron* and *Elymus.* The opportunity obtains for hybridization between races if such occurs in *Erysiphe graminis.* Wild grasses may serve as sources of primary infection and as perennial stations for overwintering races of wheat and barley mildews. These data indicate that the old concept of restriction to a host genus is untenable. Likewise the present nomenclature of specialized races may prove impracticable.

*Strains of Yellows Virus in Montmorency Cherry.* HILDEBRAND, E. M. Rasmussen and Cation (1942) have already indicated the presence of strains of cherry-yellows virus by reactions induced on peach and mahaleb cherry. However, the necrotic symptoms have not been definitely shown to be due to cherry-yellows virus because of confusion with those induced by the necrotic ringspot virus, a common contaminant in trees affected with cherry yellows. The common stunting and rosette symptom resulting from indexing from cherry to peach is now being designated as strain 1. A mosaic symptom that develops on peach seedlings with little or no stunting of growth is designated as strain 2. In the latter a variable number of leaves develop a striking mosaic pattern accompanied by leaf distortion, which is in strong contrast to the reaction induced by strain 1. Both virus strains have been found in the same orchard and in several States where the disease is present. In addition to those already indicated the possibility exists of still other strains. Whether the green ring mottle reported from Michigan, but also present in Wisconsin and New York, and symptom patterns such as chlorotic mottle and rosette are strains of cherry yellows or due to distinct viruses has not been determined with finality.

*Treating Infested Tomato Roots to Obtain Nematode Control in Greenhouses.* HOWARD, F. L. In experimental and commercial greenhouses mature tomato plant roots heavily infested with *Heterodera marioni* were treated with chloropicrin, removed after ten days, and a susceptible bean crop planted to measure results. Split plot designs of 5 and 10 replications using a 3-cc. dosage per plant in three placement patterns and two soil moisture levels were employed. The uptake of chloropicrin in the soil solution by the plants was sufficient to kill from 10 to 72 inches of stem within 48 hours. A single dose (3 cc.) caused significantly greater kill of the infested plants than divided doses (two 1½ cc., or three 1 cc.). However, in all treatments the nematodes in the cortical tissues were not killed and as the roots decayed they moved out into the soil to infest the succeeding crop. In nontreated plants the nematodes remained in the living roots and were largely removed with the intact tomato root system thus decreasing the potential inoculum. Maximum control may be obtained by removal of the undisintegrated root systems intact, by accelerating decay of the residual infested rootlets, and by then applying chloropicrin.

*Sphaceloma Causing Scab of Magnolia grandiflora.* JENKINS, ANNA E. *Sphaceloma* has been identified recently on leaves of *Magnolia grandiflora* from Georgia and Mississippi. It produces few to almost innumerable slightly raised spots on the upper side of the leaf. These may be scattered over the entire blade, or be most numerous along the midrib or in the marginal region. Spots are circular to irregular and gray with a more or less indefinite brown border. When fully developed, they often reach not more than 1.5 mm. in diameter; although, by coalescence, they form discolored areas that may involve as much as three-fourths the area of the upper leaf surface. Where the leaf is severely infected, particularly along the margin, the tissue may be killed, appearing brown on both leaf surfaces. Acervuli of the *Sphaceloma* are sparse to abundant; as seen through a hand lens they appear as small superficial black masses. The organism is described as a new species; it is suggested that it may be indigenous to the United States.

*Experiments with Eradicant and Protectant Sprays for Apple Scab Control in 1942.* KEITT, G. W., AND J. DUAIN MOORE. The floor of 40 acres on one side of a 60-acre orchard of McIntosh and other varieties was treated at bud-break with Elgetol, ½ per cent, 600 gal. per acre. Various protectant spray programs were tested on randomized, replicated, singletree McIntosh plots repeated in blocks I (ground-treated, more remote from non-ground-treated), II (ground-treated, intermediate), and III (non-ground-treated). Uncontrollable circumstances occasioned poor timing of certain applications. In block IV, situated essentially as I, similar protectant programs were used with good timing. Scab was very severe this season. Discharge studies indicate the ground treatment reduced ascosporic inoculum by 99 per cent. Counts 2 weeks after petal-fall indicate nonsprayed trees in I had 87 per cent fewer leaf lesions than nonsprayed trees in III. The protectant spray programs ordinarily included 8 applications, 3 before and 5 after bloom. In I–III the lime-sulphur spray 20 days after petal fall was omitted. In ground-treated I the percentage of scabbed fruits at harvest ranged from 11 for lime-sulphur to 19 for Kolofog (after bloom, following lime-sulphur). In non-ground-treated III the range was from 26 per cent for lime-sulphur to 77 for Kolofog. The well-timed lime-sulphur program in ground-treated IV gave 2 per cent scabbed fruit.

*A New Sugar Beet Leaf Blight Caused by a Strain of Corticium solani.* KOTILA, JOHN E. Foliage blighting of sugar beets, distinct from attack previously associated with Rhizoctonia, was first noted at Arlington Farm, Virginia, in July, 1938, and, since then, in commercial fields in Michigan, Illinois, Wisconsin, and Minnesota, incidence and severity being dependent on prolonged periods of high humidity. Heart leaves are reduced to tipburned stubs; on older leaves, necrosis may involve one-third to one-half of

the blade. The Corticium stage occurring on the dorsal leaf surface adjacent to infected tissue has basidia, sterigmata, and basidiospores ($4.82 \times 8.03$ to $8.03 \times 12.86$ μ) agreeing with *Corticium solani*. The fungus is considered a strain pathogenic to sugar beets, distinct from those previously reported. Sugar beet inoculations with pure cultures give typical symptoms on leaves, damping-off of seedlings, but no rotting of half-grown roots. Basidiospore infection occurred on 60 per cent of the sugar-beet plants exposed by placing leaves bearing the Corticium stage on screens above them. Only 1 of 58 control plants in an adjacent compartment subjected to similar atmospheric conditions (relative humidity 90 to 100 per cent, temperature 75° to 80° F.) became infected (attributed to chance air dissemination of a basidiospore). Early field infections on sugar beets probably come from the soil-borne Rhizoctonia stage, the later infections arising from basidiospores.

*Temperature as a Factor Influencing Germination of Chlamydospores of Ustilago striaeformis Forma Poae-pratensis.* KREITLOW, K. W. Chlamydospores freshly removed from smutted plants of *Poa pratensis* almost invariably fail to germinate. Earlier work demonstrated that a prolonged after-ripening period was prerequisite to successful germination of the spores. Diseased plants of *Poa pratensis* incubated at 32° C. for 16 days yielded chlamydospores that germinated 50–75 per cent. Chlamydospores removed from replicates of the same plants growing at 1–5° C., 10° C., and 25° C. failed to germinate. Chlamydospores that germinated 50–90 per cent were secured from detached smutted leaves incubated in Petri dish moist chambers for 15 to 30 days at 35° C. Similar series of detached smutted leaves incubated at 5°, 10°, 15°, 20°, 25°, and 30° C. failed to provide germinable chlamydospores despite the fact that the smutted leaves were incubated more than 30 days.

*Growth of Ustilago striaeformis Forma agrostidis in Artificial Cultuure.* KREITLOW, K. W. During the fall of 1941, 49 smutted *Agrostis alba* plants were collected from 12 widely separated pastures and examined for germinable chlamydospores. Only one collection of two plants yielded fresh, germinable spores but they consistently germinated 50–75 per cent. Single chlamydospores from this collection were germinated on potato-dextrose agar. None of the promycelia that developed from germinating spores formed sporidia. Instead, they produced lateral branches that grew and further branched until they formed a slow-growing colony. The cultures on agar were light buff in color and strictly mycelial in form. Two biotypes were differentiated on the basis of rate of growth among the cultures secured. Optimum growth of the cultures occurred at 25° C.

*Ratio of Velocity of Seedling Germination to Fungus Growth Rate As a Measure of Preemergence Damping-off.* LEACH, L. D. Tests conducted in constant temperature chambers with uniform soil moisture show that specific damping-off organisms have different temperature ranges for infection of the same host. Likewise, the same organism may differ as to optimum temperature ranges for infection of different crops. The relation between temperature and the relative severity of preemergence damping-off usually cannot be explained adequately by either the growth rates of the organism or the seedlings. The ratio of the coefficient of velocity of germination of the seedlings to the growth rate of the organism shows a close relation to the degree of infection at that temperature. In general at temperatures where the germination velocity coefficient exceeds the growth rate of the fungus, preemergence damping-off is slight or absent. When the reverse is true, infection is usually severe even with a moderate inoculum potential. The rate of post-emergence damping-off also appears to be influenced by this relation between seedling and fungus fungus growth rates. By applying this principal it has been possible to suggest planting dates for certain crops that avoid seedling infections by specific organisms. Detailed tests have been conducted with sugar beets, spinach, and watermelons and with *Pythium ultimum*, *Rhizoctonia solani*, and a species of *Aphanomyces*.

*Relation of Temperature and Soil Reaction to the Water Mold Seedling Disease of Sugar Beets.* LEACH, L. D. During the past three years sugar beets in the Sacramento-San Joaquin delta region of California have been seriously affected by a seedling disease caused by a species of *Aphanomyces*, probably *A. cochlioides*. This organism seldom causes pre-emergence damping-off but may infect a high percentage of the seedlings between emergence and thinning. Under favorable conditions many seedlings recover. Mixed infections, including *Pythium ultimum* or *Rhizoctonia solani*, are found in most fields. Seed treatments effective against *Pythium* or *Rhizoctonia* damping-off were ineffective against *Aphanomyces*. In controlled experiments infection was most severe at 20° and 25° C., moderate at 16° and 30° C., light at 12° C., and absent at 4°, 8°, and 35° C. January and February plantings were suggested as a means of avoiding infection. During 1942 no injury occurred in fields planted before February 22, but infection was severe in many fields planted during March, April, and May. Serious infestations of the beet water mold appear to be limited to soils of high moisture capacity and neutral or

slightly acid reaction. In greenhouse trials, applications of $Ca(OH)_2$ provided nearly complete control.

*Invading Plant Tumors and Their Production.* LEVINE, MICHAEL. Crown gall has occasionally been reported as showing evidence of host invasion, and, as such, has merited consideration as a malignant disease analogous to animal cancer. The present report deals with the attempts to produce such galls during the past 3 years. More than 15 species of plants grown under normal conditions in the garden were used. More than 2000 inoculations and tests were made. Surface inoculations made by lightly scratching the stem with a needle introducing a virulent strain of *B. tumefaciens* was tried. The application of pressure induced by binding tightly the inoculated area with parafilm, tape and collodion fails to induce an invasive growth. The addition of growth substances, vitamines, and vitamine-like substances applied in lanolin paste to the inoculated areas together with pressure are ineffective. Ricinus, chicory, tomato, geranium, and sunflower stems inoculated by these methods produce only surface galls. In some cases no crown galls are formed. Amputated stems inoculated with *B. tumefaciens* produce crown galls that fail to invade the tissue of the host. The puncture method alone induces tumors having the appearance of invading growths.

*Colorimetric Determination of Small Amounts of Tobacco-mosaic Virus Protein.* LOJKIN, MARY E., AND HELEN PURDY BEALE. The protein content of purified preparations of strains of tobacco-mosaic virus can be determined rapidly in a photoelectric colorimeter, using Folin's method, essentially as modified by A. M. Altschul (by communication). Colorimetric curves were obtained for 6 different strains of the virus including the type strain (*Marmor tabaci* H. var. *vulgare* H.). The effect of copper on the development of the color reaction was observed. The higher percentage of tyrosine known to be present in one of the strains, *M. tabaci* H. var. *plantaginis* H., gave a curve readily distinguishable from that of the type strain. The practical advantage of this colorimetric method lies in the ability to make quantitative determinations of virus protein when the total amount of protein available is less than 0.01 mg.

*The Determination of Particle Size of Fungicidal Materials.* MACK, G. L., AND O. A. REINKING. Particle size is the physical property of powdered fungicides that principally determines degree of effectiveness in disease control. The different expressions for designating fineness and the numerous methods of measurement have led to much confusion. The nonuniformity in size and shape of particles present in all commercial wettable sulphur and insoluble copper fungicides causes a 4- or 5-fold variation between the largest and smallest of the several average diameters used to express particle size. The shape factor is of much less importance than the size factor as the average shape of most ground fungicides approximates that of a sphere.

The particle size of 11 representative copper and sulphur fungicides was determined by 4 completely independent methods and expressed as 3 different average diameters. The Andreasen sedimentation and the air permeation methods yielded concordant results. The diameter of the particle of average specific surface appeared to be the diameter most closely related to the relative toxicity of the materials tested. Since *specific surface* is a property independent of degree of uniformity of sample and closely related to the toxicity of fungicides, this term is suggested for expressing the fineness of these powdered materials.

*Acute and Chronic Symptoms in Virus Diseases.* MCKINNEY, H. H., AND E. E. CLAYTON. Tobacco plants with severe yellow mosaic manifest an acute chlorotic-necrotic phase followed by the chronic-mosaic phase. The acute necrotic phase is limited to leaves that have attained about two-thirds their growth and less, at the time of vein clearing, whereas the chronic-mosaic phase obtains in leaves formed just before and after vein clearing. In tobacco common mosaic, acute and chronic phases are evident, but to a less degree. In tobacco ring spot, the chronic phase is less evident because symptoms are seldom apparent at usual temperatures. The persistent expression of the acute phase (necrotic spots, ring spots, and oak-leaf patterns), apparently is dependent on natural resistance that increases with age, and hinders establishment of the virus in the apical meristem. In field-grown tobacco plants entering the period of rapid growth, this resistance has been overcome and the chronic phase induced most readily by amputating 5 to 6 apical leaves ranging from 4 to 80 mm. long, and immediately wiping 4 or more carborundum-dusted leaves with highly active virus extract. In a more susceptible host (Early White Spine cucumber), this virus induces a chronic-mosaic phase at usual temperatures, and it is concluded that this is essentially a mosaic virus.

*Control of Water-core of Rutabagas by Spraying.* MACLACHLAN, J. D. The use of soil applications of borax to control water-core or brown heart of rutabagas has not

been generally adopted in south-western Ontario. The practice has not given consistent control, probably because of the high lime content of the soil. Practically complete control has been obtained by two foliar spray applications using a 2 per cent aqueous solution of borax with ¼ per cent Orvus added as a spreader. The first application was made when the roots were one to two inches in diameter; the second application approximately one month later.

*Factors Influencing the Response of Tomatoes to Sprays for Leaf-blight Control.* McNEW, GEORGE L. Tomato defoliation has been effectively prevented in western New York by 4 applications of copper fungicides. Yields were increased by 1 to 6 tons per acre and the amount of U. S. No. 1 fruit by 10 to 45 per cent, depending upon: the severity of infestation by *Macrosporium solani* and *Septoria lycopersici;* the effectiveness and phytotoxicity of the fungicides applied; the timeliness of the spray applications; the amount of fruit injured by sunscald, fruit blight (*Phytophthora infestans*) and Anthracnose (*Colletotrichum phomoides*); and the fertility of the soil. Data from replicated plots receiving 9 different fertilizer treatments showed that the increase in yield from spraying or dusting was directly correlated with the effectiveness of the fertilizer in promoting plant productivity. None of the fertilizers or side dressings prevented defoliation on unprotected plants. In one commercial-scale test, the standard spray schedule employing either Bordeaux mixture 4–2–50 or insoluble copper completely eliminated Phytophthora blight that destroyed 44 per cent of the fruit and reduced yields by 4 tons in the unsprayed controls. The copper compounds were only partially effective against anthracnose, but Fermate reduced the amount of diseased fruit in two fields from 32 and 16 per cent to less than 2 per cent.

*Relative Effectiveness of Organic and Inorganic Fungicides as Seed Protectants.* McNEW, GEORGE L. At least 3 organic fungicides offer promise as substitutes for the copper and mercury compounds now used to protect vegetable seeds. Tetrachloro-para-benzoquinone (Spergon), ferric dimethyldithio-carbamate (Fermate), and tetramethylthiuram-disulfide (Thiosan) have effectively protected seed from decay by *Pythium ultimum* in greenhouse tests and have given excellent results under field conditions. These organic fungicides are the only ones suitable for Lima beans, and they appear superior to the metallic treatments on peas. Sufficient data have been obtained to recommend Spergon for peas and Lima beans. The other two organics are only slightly less effective in promoting emergence and increasing yields. Further tests will have to be made with Fermate and Thiosan on these two crops and on spinach and sweet corn, where they usually have been as effective as metallic compounds. In replicated tests with 9 treatments on sweet corn in 1942, Thiosan gave the largest increase in yield (20%) followed by Semesan Jr., Barbak C, and Spergon. The treatment of pea seed increased yield by preventing loss of seedlings from preemergence seed decay and reduction in plant vigor from post-emergence seed decay. Spergon has a slight advantage over other fungicides in its ability under some conditions to stimulate plant growth in the absence of disease.

*Relation of Dosage of Certain Copper Sprays to Copper Injury and Leaf-spot Control on Montmorency Cherry.* MILLER, H. J. Dosages of Basicop, Tennessee "26," Cupro-K, and Bordow were varied by applying each at concentrations of ⅛, ¼, ½, and 1¼ lb. of actual copper to the hundred with 3 lb. hydrated lime in 3 applications before harvest and 1 after. A copper ring injury on fruit was found to increase with increasing concentrations of copper and the ½ lb. rate caused 55.4, 46.6, 36.9, and 26.4 per cent injured fruits, respectively, with these materials. The ring injury, heretofore underscribed, occurred as a black line on the fruit adjacent to the base of the stem, completely encircling it in many cases, and was found very objectionable in the canned product. Tennessee "26" and Bordow gave better control of leaf spot than Cupro-K and Basicop at the lower rates. Control was correlated with increasing concentrations of all 4 materials. At ½ lb. all materials, except Basicop, had reached approximately maximum control (better than 70 per cent). Basicop required 1¼ per 100 to reach the same point. Size, color, and sugar content of fruit showed no relation to concentration of copper.

*Catex, A Replacement for Formaldehyde in the Control of Onion Smut.* NEWHALL, A. G., and WILLIAM R. RADER. Greenhouse and commercial field tests the past season have demonstrated the effectiveness of Catex as a possible substitute for formaldehyde in the drip method of controlling onion smut. This product, resulting from the destructive distillation of Southern pine stumps in the manufacture of naval stores, contains certain phenolates that presumably are responsible for its fungicidal properties. In 9 farm tests, where the mean percentage of smut was 25.5 in the check plots, formaldehyde reduced it to 3.3, while Catex reduced it to 3.9 per cent. Mean yield increases over checks on 7 of these farms amounted to 35 per cent and 43 per cent for Catex and formaldehyde, respectively. The Catex solution was employed in the same manner, with the same equip-

ment as that used in applying formaldehyde. Two gallons of Catex in 100 gallons of water applied to an acre (37,000 ft. of row) were equivalent to 1½ gallons of formaldehyde. Doubling the concentration of Catex gave no appreciable increase in effectiveness and sometimes retarded germination slightly. Present quotations indicate that replacement of formaldehyde by Catex would not increase the cost of controlling onion smut.

*Uromyces betae in Canada.* NEWTON, MARGARET, AND B. PETURSON. Beet rust (*Uromyces betae*) was first observed in Canada in 1935 when it occurred on sugar beet plots at two stations in British Columbia, *i.e.*, Saanichton, Vancouver Island, and Agassiz on the mainland, places 90 miles apart and separated by 35 miles of water and a high mountain range. The plots at both stations were sown with seed imported from Europe and carrying large numbers of urediospores and teliospores. Since 1935, this rust has been present each year in the Saanichton district, where it attains greatest severity in early spring and late autumn. Greenhouse studies showed that *U. betae* is extremely sensitive to high temperature. The optimum for urediospore germination is 10° to 22° C. and for rust development 15° to 22° C. Varieties susceptible at from 15° to 22° C. developed resistance at higher temperatures, becoming extremely resistant at 26° C. The sensitivity of this rust to high temperatures probably accounts for its absence in Alberta, Saskatchewan, and Manitoba, as in these Provinces the summer temperature is probably too high for its development.

*Influence of Certain Soil Amendments on the Yield of Cotton Affected by the Fusarium-Heterodera Complex.* PINCKARD, J. A., O. A. LEONARD, AND H. C. MCNAMARA. Cotton wilt, caused by the combined action of *Fusarium vasinfectum* and *Heterodera marioni*, has been substantially reduced by deep applications of nitrogenous organic matter on two soil types. A compost of spoiled alfalfa hay, 8 tons per acre, buried deep in the furrows in January, increased the 3-year average acre yield of seed cotton 689 lb. Stable manure increased the yield 617 lb., but, when applied broadcast, it was equivalent only to 4 tons buried deep in the furrows, the increase being 500 lb. Eight tons of dry sagrain (*Sorghum vulgare*), enriched by 30 lb. of nitrogen as ammonium sulphate, increased the yield 343 lb. Cyanamid, equivalent to 30 lb. of nitrogen, increased the yield 301 lb. more than the untreated plots, which gave an average total yield of 933 lb. per acre per year. On similar disease-free soils the above application of cyanamid is a recommended practice and yields of 2,000 pounds are common. Disease distribution maps, made in 1939 and 1942, show no reduction in the incidence of either disease. (Cooperative investigations of the Division of Cotton and other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Mississippi Agricultural Experiment Station.)

*Dissemination of Phytomonas poinsettiae on Greenhouse-forced Poinsettia.* PIRONE, P. P. Further investigations on the new bacterial disease of poinsettia (*Phytomonas poinsettiae*) show the organism transmitted by infested propagating knives to cuttings and to the stubs remaining on the stock plants. The great majority of cuttings taken with infested knives either fail to root and eventually rot, or root less profusely than those taken with clean knives. The knives were contaminated in two ways, by rubbing them with bacteria obtained from pure cultures, and by passing them through diseased stems. Cuttings taken from diseased stock plants may carry the organism, provided the cuts are made within an inch of stem lesions. The disease also was transmitted experimentally by rubbing contaminated fingers over leaves just enough to break the epidermis and by spraying with a bacterial-water suspension leaves previously injured by needle pricks. No infection was observed when uninjured leaves and stems were sprayed with bacteria. Secondary (leaf) infections probably occur from strong syringing of the plants. The principal source of inoculum for such infections is the tiny, glistening, orange masses of bacteria that ooze to the surface in or near stem lesions. The causal organism can survive for at least 6 months on bacterial agar slants, and for at least one year in the stems of diseased plants.

*Effect of Site of Inoculation upon Severity of Curly Top in Tobacco.* PRICE, W. C. Transmission of sugar-beet curly-top virus to healthy tobacco by grafting with cions from tobacco plants that had recovered from curly top resulted in mild symptoms in some instances, severe in others, and intermediate in still others. Similar results were obtained when recently infected plants were used as sources of cions. Thus, there seemed to be no definite correlation between severity of symptoms in grafted plants and stage of infection of plants from which cions were taken. Moreover, severity of symptoms was not found correlated with age or growth rate of the grafted plants at the time symptoms first appeared. A correlation was found, however, between severity of disease and site of inoculation when the virus was transmitted by the insect vector, *Eutettix tenellus*. Tobacco plants infected by allowing viruliferous insects to feed on the terminal bud and

adjacent leaves invariably developed severe symptoms, but those infected by insects feeding on a single leaf or a 2-inch section of stem well below the terminal bud frequently developed only mild symptoms.

*Control of Damping-off of Nursery Pine Seedlings.* RIKER, A. J., R. H. GRUENHAGEN, AND L. F. ROTH. The forest-nursery practice common in Wisconsin of using approximately 2 per cent sulphuric acid to control damping-off of pine seedlings often provides (1) trouble in application, (2) toxicity to seedlings and (3) an unfavorable effect on soil nutrients. Greenhouse and outdoor trials in 3 Wisconsin forest nurseries have been made in 6 years mostly with red pine to find an adequate control having fewer disadvantages. Among 26 substances tested repeatedly, best results came from a combination seed dust with mercuric phenyl cyanamide and cadmium oxide (used primarily against Pythium) plus a seed-level soil application of $\frac{3}{4}$ oz. per 4 by 12 ft. seed bed of calomel (used primarily against Rhizoctonia). In large-scale nursery tests during the last 3 years, made in comparison with sulphuric acid, the combination seed dust and soil treatment showed similar control and germination, negligible injury, and no apparent nutritional disturbance. Because of the mercury shortage, tetramethyl thiuramdisulphide was tried last season in 3 nurseries. When about $\frac{3}{4}$ oz. per 4 by 12 ft. seed bed was applied 3 days before seeding, it was comparable to the combination treatment with respect to ease of application, negligible injury, high germination, and commercial control. In addition it killed many weed seeds.

*White Pine Selections Tested for Resistance to Blister Rust.* RIKER, A. J., T. F. KOUBA, W. H. BRENER, AND L. BYAM. A white-pine blister-rust area has been established in a well-isolated location in central Wisconsin and provided with irrigation. Seedlings and veneer grafts from over 150 individually selected white pines are being tested by both natural and artificial inoculation. The selections were made because they showed no blister rust after 15 to 20 years' natural exposure to infected Ribes. A graft and some seedlings from each of the selected trees were planted in a series of 4 by 12 ft. beds. *Ribes cynosbati, R. missouriense,* and *R. nigrum* were placed between uine beds so that each pine tree was 3 to 6 feet from a Ribes bush. About 1,000 pine grafts and 10,000 pine seedlings have been planted. With 2 of 4 replications natural inoculation was supplemented with artificial inoculations by fastening a telia-bearing leaf on the top of each pine. Then each bed was covered with a muslin cage and kept moist for $2\frac{1}{2}$ days. Among progeny from the first 63 selected trees, both naturally and artificially inoculated in September 1941, about 5 per cent of the grafts and, with certain selections, 100 per cent of the seedlings showed infection in the fall of 1942. As high as 50 per cent of cuttings without hormone treatment and made from commercial 4-year-old white pine seedlings have been rooted in cold frames.

*Genetic Studies of Certain Mutant Characters in Venturia inaequalis.* SHAY, J. R. Several cultural mutants carry factors for ascospore abortion. Abortion may be complete (with no differentiation of the spore observable) or partial (with development of uni- or bicellular, misshapen, colorless, sometimes viable, spore-like structures) for the ascospores bearing these factors, depending upon the mutant concerned. Three mutants that arose as white or pinkish-white sectors in culture transmit factors for ascospore abortion. In 114 asci studied from a cross involving one of these, *white* (W), *normal* (w), the aborted ascospores (4) of any ascus when viable consistently gave rise to *white* cultures, whereas the normal ascospores (4) gave *normal* cultures. This indicates that ascospore abortion and white color result from the same mutation. *White* (W) was located with respect to the centromere and found to be associated in the same chromosome arm with and proximally to another mutant, *tan* (T), which gives 8-spore asci in matings with *normal* (t) and is tan in color. Another mutant *nonconidial* (Nc) was located in relation to the centromere, but probably is not linked with *tan*. The sex factor appears to be located at a considerable distance from the centromere and is probably not linked with *white*.

*Control of Granville Wilt (Bacterium solanacearum) of Tobacco and other Plants by Applications of Urea to the Soil.* SMITH, T. E., AND E. E. CLAYTON. In tests with tobacco grown on naturally infested soil, applications of urea were made in the fall and spring at 3 rates. Treatments were made in triplicate on 3-row plots 109 feet long. The material was broadcast on the surface and disked into the soil to a depth of about 6 inches. Counts of wilted plants were made on the center row of each plot. Following applications made October 17, 1941, at the rates of 250, 500, and 1000 lb. per acre of "Uramon" (42 per cent N), 80, 68 and 13 per cent, respectively, of the tobacco plants were wilted by August 1, 1942. Following applications made on March 24, 1942, at these rates, 49, 34, and 3 per cent, respectively, of the tobacco plants were wilted by August 1, 1942. In another experiment, tobacco, tomato, Irish potato, egg plant, pepper, petunia, black nightshade, and castor bean were grown on infested soils. All plants of the more susceptible

species were wilted by August 1 on the nontreated plot. "Uramon" applied at the rates of 500 and 1000 lb. per acre, 10 weeks before planting, effectively controlled wilt on all species included in the test. The 1000 lb. rate almost completely eliminated the disease. —Cooperative investigations of the Bureau of Plant Industry, U. S. Department of Agriculture, the N. C. Agricultural Experiment Station, and the N. C. Department of Agriculture.

*Laboratory Assay of the Effects of Wetting Agents on Organic Soil-sterilizing Solutions.* SORRELL, M. B., AND F. L. HOWARD. The toxicity of chloropicrin, formaldehyde, 1,1 dichloro nitroethane (Ethide), carbon disulphide, and ethylene dichloride to sclerotia of *Sclerotium rolfsii* was compared first without and then with wetting agents. Graphs indicate that exposure time plotted against concentration of toxicant place the chemicals in the above respective order on the basis of lethal dose necessary to kill 50 per cent of the sclerotia. A numerical *toxicity factor* may be assigned to each chemical in solution on the basis of exposure time and toxicant concentration to give "L.D.50." Lowering the interfacial tension of the sterilizing solution increases the penetration and the effectiveness of the treatment, except where the wetting agent reacts with the toxicant. The action of the wetting agents (Santomerse #3, Vatsol OT, Penetrator W1495, Triton NE) was determined on the basis of depression of interfacial tension ("Contact angle" and "Draves" test), and of the physical or chemical compatibility of the wetting agents with the toxicants. Each wetting agent may cause an increase or decrease in the value of the toxicity factor, and thus affect the killing power of the toxicant.

*Regional Spread of Wheat Stem Rust from Barberry-infested Areas of the Virginias in 1942.* STAKMAN, E. C., R. U. COTTER, AND W. Q. LOEGERING. There is strong circumstantial evidence that widespread, although not destructive, infection of stem rust on wheat in Ohio and contiguous areas westward and northwestward resulted from inoculum carried by the wind from barberry-infested areas of Virginia and West Virginia, where there was abundant rust development extending outward from barberry areas. Considerable numbers of urediospores were carried into Ohio and westward to Illinois by southeast winds from June 10 to 12, inclusive; extensive development of rust followed about 10 days later; and the concentration of race 38 in Virginia, West Virginia, Pennsylvania, Ohio, Indiana, Michigan, and Illinois was so conspicuous as to indicate clearly the original source of the inoculum. (Cooperative investigations between the U. S. Department of Agricultural and the Minnesota Agricultural Experiment Station.)

*Adsorption of Chloropicrin and Other Fumigants by the Soil.* STARK, FRANK L., JR. By means of a spring type microbalance the weight of chloropicrin and other soil fumigants adsorbed by soil particles was determined. The amount of gas adsorbed is dependent primarily on size of the soil particles, soil temperature, and concentration of gas. Heavy soils having a large clay fraction may adsorb chloropicrin up to 10 per cent of their weight from a saturated atmosphere. Degree of aggregation of soil particles has little influence on either the quantity of gas adsorbed or rate of adsorption. Carbon bisulphide is taken up in smaller quantities than chloropicrin and at a much slower rate than either chloropicrin or formaldehyde. Formaldehyde is adsorbed to a lesser extent than either chloropicrin or carbon bisulphide but at about the same rate as chloropicrin. Adsorption probably impairs the efficiency of volatile fumigants by reducing the concentration and the diffusion of vapor in the soil, especially in the case of chloropicrin. Because some soil fumigants are more readily adsorbed than others, the efficiency of one chemical may be impaired more than another when a heavy soil is treated, which may account for the differences obtained in investigations on the relative effectiveness of various soil fumigants.

*A Study of Claviceps on Zizania.* STEINMETZ, F. H., AND CHARLES M. WRIGHT. A species of *Claviceps* has been observed on *Zizania* in the vicinity of Orono, Maine. Studies were undertaken to obtain information on the overwintering, development, and spread, and to determine the possible relation to ergot on other hosts. Storage in moist sand under out-of-door conditions or storage in water in unsealed jars at 4° C. preserved the viability of the fungus. Favorable storage for 30 to 60 days was followed by ascocarp development when cultured under greenhouse conditions. Conidiospores from early field infections or from mature sclerotia were effective in cultures. Inoculations with conidia from infected wild rice to greenhouse-grown barley, oats, wheat, and millet were unsuccessful. Inoculations with conidia from infected *Festuca, Calamagrostis, Agropyron,* and *Dactylis* to greenhouse-grown *Zizania* were unsuccessful. Conidia from *Zizania* to *Zizania* were successful. Based upon determinations involving 1200 plants in the field it was found that 0.82 per cent of the pistillate flowers were infected. Morphological studies indicate that the *Claviceps* on *Zizania* is different from *Claviceps purpurea.* In

the field *Oscinella neocoxendix*, a dipterous insect, appeared to be chiefly responsible for secondary spread of the fungus.

*A Simple Rapid Method of Field Testing Fungicides on Apples.* STODDARD, E. M., AND J. W. HEUBERGER. This method makes use of young standard trees planted 10′ × 10′, or 435 to the acre, which is at once economical of land and labor. Much time and labor are saved in the spraying operations, which can be done with hand equipment. Such a planting of young trees permits simultaneous testing of many materials. Data on control, injury, dosage, timing, or any other particular can be obtained more easily than on large trees. Proper randomization and replication of plots is readily accomplished. Uniform disease infection can be assured by uniform distribution of inoculum, such as overwintered scab-infected apple leaves. In the case of apple scab, data taken over a period of years show a direct correlation between control on foliage and control on fruit. Thus, fungicides are rated in the same order by leaf data as by fruit data on control of apple scab. If fruit is necessary to the experiments, dwarf trees can be substituted for the young standard trees. This method would be applicable also to the testing of materials on other leaf diseases of fruit trees, such as leaf spot on cherry, *Bacterium pruni* on peach, and cedar rust on apple.

*The Use of Ammonia in Controlling the Truffle Disease of the Mushroom.* STOLLER, B. B. The spores of *Pseudobalsamia microspora* were exposed 24 to 48 hours to 19 fumigants. Of these only ammonia, dimethylamine, quinoline, and methanol retarded the germination of the spores 4 months or longer. The fumigants usually employed in mushroom houses, formaldehyde and sulphur dioxide, had no retarding effect on spore germination. Ammonia effectively retarded spore germination at a concentration of 0.3 g. per liter of air, using 75-ml. bottles in which the spore mass and adhering compost was large compared to the size of the container; but half of this concentration sufficed in the fumigation of empty mushroom houses, since the spore mass was comparatively small. Spore germination also was retarded for 4 months or longer, with few exceptions, by the final fermentation or "sweating out process"; a strong ammonification of the compost is characteristic of this process. The high temperatures attained during this process had no effect on spore germination in the absence of a strong ammonification—that is, under anaerobic conditions or in the acid condition of the compost produced by the mushroom mycelium. It then may be inferred that the ammonia volatilizing during this final fermentation prevents or retards the germination of these spores.

*A Defense Mechanism for Resisting Disease in the Mushroom.* STOLLER, B. B. The growing mycelium of *Agaricus* (*Psalliota*) *campestris* was observed to produced a volatile substance with strongly oxidizing properties. A comparative study of the reactions of this substance, using oxidase or oxidizing reagents like guaiacum gum and benzidine, with that of o- and p-benzoquinone, hydrogen peroxide, and chlorine, suggests that it is in the nature of a quinone or "activated" peroxide. This substance readily diffused from the mycelium into an agar medium, and produced characteristic color changes in some 100 organic compounds added to the agar. Two pathogens of the mushroom sporophore, which failed to grow on the mushroom mycelium, did not produce sulphides, whereas a pathogen of the mushroom mycelium did. The sulphides initiated by the growth of the latter suppressed the volatile substance of the mycelium, and decolorized many of the color reactions in the agar medium; hydrogen sulphide *in vitro* reacted similarly. The oxidizing substance volatilizing continuously from the mycelium-permeated compost is suggested as serving as a defensive mechanism; only those of its enemies that produce sulphides can attack the mushroom mycelium.

*Relation of the Concentration of Copper Fungicides to Disease Control and Spray Injury on Grapes.* SUIT, R. F. Concentrations of Bordeaux as low as 2–2–100 controlled *Guignardia bidwellii* and *Uncinula necator*, and as low as 3–3–100 controlled *Plasmopara viticola* in tests made the past 3 years. The standard spray schedule of 5 applications for black rot control was reduced to 3 without affecting control. The after-bloom application was the most important. Eight insoluble coppers used at the copper equivalent of Bordeaux 4–4–100 and 2–2–100 gave variable control. The majority were satisfactory but inferior to Bordeaux. All insoluble coppers caused injury at the higher concentration. The injury was characterized by smaller, yellowish leaves, reduced vine growth, and reduced yield. Yield reduction was greater the year following the injury without further spray applications. The injury was less apparent in a dry season. The insoluble coppers were not injurious when used at concentrations not exceeding ½ lb. of metallic copper per 100 gal. or when used at higher concentrations with 1 lb. of lime for each ¼ lb. of metallic copper. The addition of spreader-stickers increased the effectiveness of Bordeaux and the insoluble coppers without increasing the phytotoxicity of the insoluble coppers. Fermate gave commercial control of *Plasmopara viticola*, but was not so good as the copper fungicides, and did not control *Uncinula necator*.

*Tetramethyl thiuramdisulphide and ferric dimethyl dithiocarbamate as Seed Protectants on Vegetables.* TAYLOR, CARLTON F., J. A. RUPERT, AND J. G. LEACH. Tetramethyl thiuramdisulphide and ferric dimethyl dithiocarbamate, each of 80 per cent concentration of active ingredients, have shown promise as seed-treatment materials on a number of vegetables. In preliminary tests at maximum adherence loads they proved as good as Cuprocide on carrot, muskmelon, pea, spinach, sweet corn, and tomato. These tests were conducted in greenhouse loam at pH 5.2 to 5.4. On spinach, at dosages ranging from 0.1 per cent by weight to maximum adherence, both of these materials have proved superior to Cuprocide (red) at 2 per cent dosage in the prevention of both preemergence and post-emergence damping-off. Tetramethyl thiuramdisulphide and ferric dimethyl dithiocarbamate were outstanding in their protection against post-emergence damping-off. There was some evidence of injury on lettuce as indicated by reductions in emergence. Injury was greatest at maximum adherence loads and least when the dosage was reduced to 0.5 per cent with tetramethyl thiuramdisulphide and 1.0 per cent with ferric dimethyl dithiocarbamate. At these dosages emergence averaged 86 and 87 per cent, respectively, as compared with 95 per cent with Cuprocide and 39 per cent with the nontreated check.

*Cellular Studies in Relation to Rust Resistance.* THATCHER, F. S. Further investigation has been made of the observation that susceptibility to rusts is associated with local permeability increase of host cells, and that resistance is associated with decreased permeability. These relationships have been corroborated in the instance of the "X-reaction," where extremes of susceptibility and resistance may be present on a single leaf infected with only one rust race. The "browning" reaction, involving a change from initial susceptibility to a belated appearance of resistance, is associated with a progressive and extreme permeability increase induced by high temperatures. A previous hypothesis relating to the mechanism of rust nutrition seemed to require that the parasite be wholely extra-cellular in so far as the host plasma membrane is concerned. This requirement is established as a fact. Resistance of Hope wheat in the mature-plant stage may be partly determined by lack of availability of water to the parasite. An hypothesis is offered to explain the cause of different degrees of permeability change associated with different rust reactions.

*Studies on Experimentally Produced Physiologic Races of the Oat Smuts.* UTTER, L. GORDON. Race 1 of the loose smut (*Ustilago avenae*) of oats gives high infections on Gothland variety but seldom infects Monarch, while Race 1 of the covered smut (*U. levis*) infects Monarch but not Gothland. Hybridization of these races has resulted in 40 collections of smut having been selected on infection behavior over a period of years. Twenty-four collections were of the covered smut type, but they attacked Gothland, while Monarch was resistant to most of them. Sixteen of the selected collections resembled the loose smut, except that they infected Monarch more frequently than Gothland. On infection results, 2 collections of covered and 4 of loose smut appeared to be segregated parental smut types. Infection data for the past 4 years have confirmed earlier determinations of many of these collections as physiologic races. Thirteen races of covered smut were separated on 8 varieties of oats, including Gothland and Monarch. Similarly, 9 loose smut races were separated on 6 oat varieties. In both the loose and covered smuts, certain collections behaved identically on those oat varieties that distinguished the above races. Evidence exists that these collections can be further separated into distinct physiologic races by using additional oat varieties.

*The Relative Positions of the N and N′ Factors on Nicotiana tabacum Chromosomes.* VALLEAU, W. D. *Nicotiana glutinosa* contains a factor N that causes inoculated plants to react to all strains of the tobacco-mosaic virus so far tested with local necrotic spots. Most varieties of *N. tabacum* mottle when inoculated with these strains. Many varieties of *N. tabacum*, however, contain a factor N′, which causes them to react to a few strains of tobacco-mosaic virus with a necrotic reaction closely similar to the *N. glutinosa* reaction. Other varieties (n′n′) react to the same strains by mottling. To determine whether the N and N′ factors are borne on homologous chromosomes or on different chromosomes, a cross was made between NN and N′N′ Burleys. An $F_1$ plant was then selfed, backcrossed with NN, and backcrossed with N′N′. Inoculation of the 3 progenies with an ordinary strain of tobacco-mosaic virus gave ratios nearly identical with those expected if the factors were carried on homologous chromosomes. It is probable, therefore, that the N factor in *N. glutinosa* and the similar factor in *N. tabacum* are allelomorphs.

*Forms and Concentrations of Nitrogen Utilized by Phytomonas solanacearum.* VAUGHAN, E. K. In numerous tests the tomato bacterial-wilt organism grew poorly on media containing only nitrate nitrogen and fairly well when supplied with ammonia nitrogen, but was much better able to utilize organic forms of the latter. When grown on media made from tomato plant sap containing less than 360 or more than 600 p.p.m.

of nitrogen the bacteria grew rather poorly. On media made from sap having 365 to 600 p.p.m. of nitrogen, growth was good and was proportional to the total amount of nitrogen, rather than the amount of nitrate nitrogen present. When grown on synthetic media containing only carbohydrates and nitrogenous compounds, concentrations of nitrogen greater than 280 p.p.m. caused a reduction in the growth of the bacteria, while growth on media made with balanced nutrient mixtures was best when the concentration of nitrogen was from 300 to 500 p.p.m.

*Tobacco Anthracnose—A New Plant-bed Disease in Maryland.* WALKER, E. A. Anthracnose (small pox) has caused serious losses to tobacco seedlings in Maryland plant beds during the 1941–1942 seasons. Small plants were killed and larger ones showed distorted and spotted leaves. Cankered spots appeared on the main stem and the leaf midrib. Anthracnose is favored by wet weather at which time it spreads rapidly over the plant beds. It appeared in the field in 1942 on the leaves, stems, flower heads, and on suckers after the crop was harvested. *Colletotrichium* sp. and *Gleosporium* sp. have been isolated from lesions on infected plants. Spergon and Bordeaux-mixture sprays have given some control.

*Survey of Pea Diseases in Wisconsin in 1942.* WALKER, J. C., AND W. W. HARE. The survey was made in 654 fields comprising 4714 acres, well distributed as to season of planting, geographical location, and variety. Major diseases were root rot (*Aphanomyces euteiches*), anthracnose (*Colletotrichum pisi*), Ascochyta blight (*Mycosphaerella pinodes*), bacterial blight (*Bacterium pisi*), near-wilt (*Fusarium oxysporum* f. *pisi race 2*), mosaic, and streak. Root rot was severe in 28 per cent of all fields, causing an over-all loss to the entire acreage of around 10 per cent. A trace or more of bacterial blight, ascochyta blight and anthracnose was found in 48, 45, and 38 per cent, respectively, of all fields examined, while they were moderate to severe in 12, 6, and 9 per cent, respectively. Evidence shows that the 3 pathogens were introduced generally with western-grown seed. A definite correlation between severity and length of interval between pea crops was noted for root rot, anthracnose, and Ascochyta blight. 95 per cent of the acreage was planted with wilt-resistant varieties and no case of wilt (*F. oxysporum* f. *pisi race 1*) was found. Near-wilt, mosaic, and streak were important chiefly in fields planted to reach the canning stage during the last 10 days of the season.

*The Relation of Host Nutrition to the Development of Cabbage Yellows.* WALKER, J. C., AND W. J. HOOKER. A strain of cabbage very susceptible to yellows (*Fusarium oxysporum* f. *conglutinans*) was grown in quartz-sand cultures irrigated by the continuous drip method with Hoagland's solution adjusted to concentrations of balanced nutrient at 0.1, 0.5, 1, 2, and 3 times the basal solution and to high and low concentrations of one of the following: K, P, or N. The rate and extent of disease development was highest at the basal concentration or 0.5 basal. They were less at 0.1 basal, and were particularly retarded at 2 and 3 times basal. Reduced potassium consistently increased disease development. Reduced phosphorus and reduced nitrogen sometimes, but not consistently, reduced disease development. Changes in the latter were not clearly correlated with changes in rate of growth of the host. The same general results were secured when disease development was retarded either by reduced sand temperature or by use of a moderately resistant host variety. A field experiment showed decrease in disease index with increase in fertility level.

*Effect of Temperature and Relative Humidity on Occurrence of Blossom Blight of Stone Fruits.* WEAVER, L. O. *Sclerotinia fructicola* infected blossoms of potted peach trees in a saturated atmosphere within 18 hours at 10° C., 8 to 12 hours at 15° C., 11 hours at 20° C., and 5 hours at 25° C. At each temperature, the greatest percentage of blossom blight occurred at high relative humidities (80 and 90 per cent). The entire blossom is blighted when any one of the flower parts is infected at a relative humidity above 90 per cent. At lower humidities, blossoms were most frequently blighted from stamen infection. Peach blossoms in the pink stage were not so readily blighted as open blossoms, but infection may be secured in high humidities. Varieties of peach with large petals were not so readily destroyed as those with small petals, which have the essential organs exposed. Closed blossom buds usually were not blighted. Blossoms exposed to infection 5 days after opening were infected only after prolonged periods in saturated atmosphere or when held at 90 per cent relative humidity. At lower humidities, the infected flower parts dry and drop so that the developing fruit is not infected. The effects of temperature and relative humidity on germination of conidia and growth of mycelium are closely correlated with infection of blossoms.

*Correlations of Laboratory and Greenhouse Methods of Testing Fungicides.* WELLMAN, R. H., AND S. E. A. MCCALLAN. A high correlation in control by 400 heterogeneous

compounds exists between the greenhouse diseases, late blight, septoria leaf spot, and early blight (see McCallan and Wellman, demonstration abstract). A positive correlation exists between the average laboratory fungistatic LD50 value for 4 fungi, differing from those causing the foliage diseases, and the average control obtained for the 3 foliage diseases. No higher correlation was obtained when the same fungus, *Alternaria solani*, was compared in laboratory and greenhouse. This correlation is lower than that between the different diseases or between different fungi in the laboratory. A few compounds with laboratory LD50 values between 1,000 and 10,000 p.p.m. controlled disease as well as Bordeaux mixture with an LD50 of about 10 p.p.m. Also, some compounds whose laboratory toxicity was 10-fold greater than Bordeaux mixture failed to control disease. Where tenacity was not a factor, 32 compounds were grouped alike on the basis of control in limited field tests and in the greenhouse by these 3 foliage diseases. With 178 compounds the correlation between wheat smut and these foliage diseases was comparable to that between foliage diseases and laboratory tests and much better than between wheat smut and laboratory tests.

*The Toothpick Method of Inoculating Corn for Ear and Stalk Rots.* YOUNG, H. C., JR. A simple and efficient method has been devised for increasing inoculum and for testing the reaction of lines of corn to fungi causing ear rots and stalk rots. Quill-type wooden toothpicks are boiled in water to render them non-injurious to fungal grwth; they are then placed in 2 cc. of potato-dextrose broth in test tubes, after which the tubes are sterilized and inoculated in the usual manner. After the fungi have made requisite growth the tooth picks are inserted directly into young corn tissues, but older tissues must first be punctured with a sharp metal instrument. This method has several advantages: a uniform amount of inoculum is introduced into the plants; different sections of the stalk or ear can be inoculated with the same or different organisms at one time; the point of inoculation is detected readily; the spread of the pathogen from the peg is easily traced; and large populations can be inoculated rapidly. Selfed lines of corn thus inoculated differed markedly in reaction to *Diplodia zeae, Gibberella zeae,* and *Helminthosporium* sp.

*Purple-top Wilt of Potatoes Caused by the Aster Yellows Virus.* YOUNKIN, S. G. Further evidence has been obtained indicating that the eastern strain of the aster-yellows virus may cause purple-top wilt of potatoes. Extensive tests have demonstrated that *Macrosteles divisa* transmits a strain of the aster-yellows virus from naturally infected *Ambrosia artemisiifolia* to potato. The symptoms induced are typical of those found in naturally infected plants. Preliminary experiments indicate that insect number may be a factor limiting successful transmission of the aster-yellows virus to potato. In tests involving insect populations where a minimum of 95 per cent of the individuals were infective, 10 insects per plant gave a significantly higher incidence of infection than 5, whereas 20 insects appeared to transmit the virus no more effectively than did 10. In greenhouse tests Green Mountain was less susceptible than Katahdin and Smooth Rural. Approximately 200 grafts were made on *Nicotiana rustica* using cions from naturally infected, purple-top plants of the varieties Katahdin, Sebago, Rural, and Cobbler from 18 localities in New York and Pennsylvania. In 4 cases virus transmission to *N. rustica* was obtained. The symptoms of these 4 recovered strains were identical on *N. rustica,* but distinct from the symptoms induced by the ambrosia strain. On asters all strains appeared similar.

*Genetic Studies of Symptom Expression of Bean-Mosaic Virus 4.* ZAUMEYER, W. J., AND L. L. HARTER. Bean-mosaic virus 4 was previously described as a new virus disease of beans. It produces local lesions in some varieties and systemic infection in others. The plants that showed local lesions, although susceptible to the virus at the points of inoculation, were immune from systemic infection. The virus was recovered from leaves showing local lesions but not from any any other part of the plant. All plants that did not show the local-lesion type of infection developed systemic mottling about 10 days after inoculation. Results demonstrated that in the hybrids investigated, the inheritance of the expression of symptoms of this virus was governed by a single genetic factor difference and that local-lesion expression was dominant to systemic infection of the virus. The reaction of the heterozygous plants was indistinguishable from that of plants homozygous for the local necrotic type of infection. Although immunity from bean-mosaic virus 4 has not been noted, varieties possessing the dominant gene for virus localization are considered commercially resistant, since in these varieties the infection does not become systemic, and little or no damage to the plant results.

*Internal Therapy with Organic Chemicals in Treatment of Vascular Diseases.* ZENTMYER, GEORGE A., JR., AND JAMES G. HORSFALL. Evidence was obtained in 1941 and 1942 that organic chemicals, including in particular certain reducing agents, when intro-

duced into the vascular system of plants, have an ameliorating effect on wilting and disease advance in several vascular diseases. Randomized experiments using 9 chemicals and 5 chemotherapeutic methods on 800 verticillium-infected eggplants showed that wilting was reduced by injections of 8-hydroxyquinoline sulphate, hydroquinone, ascorbic acid, and 8-hydroxyquinoline benzoate, and by soil treatments with 8-hydroxyquinoline sulphate on sandy-loam soil. In injection experiments in 1942 on 500 elms 6 to 10 feet tall, quinone, pyrogallol, p-nitrophenol, 8-hydroxyquinoline sulphate, and hydroquinone again reduced the severity of Dutch elm disease. Some of these chemicals have been shown to inactivate the toxin of *Ceratostomella ulmi in vitro*. Results on 70 verticillium-infected maples, 34 of which were injected with 8-hydroxyquinoline sulphate in 1941, showed that 56 per cent of the treated trees and 28 per cent of the checks had improved or shown no further decline in 1942. Toxins are primary factors in these diseases. Reduction in wilting and retardation of disease may be the result of inactivation by the chemicals of free toxin in the plant. It is also possible that the chemicals may directly reduce the toxin-induced vascular plugging.

## ABSTRACTS PERTAINING TO DEMONSTRATIONS

*Different Vector Specificities for Varieties of a Plant Virus.* BLACK, L. M. Experiments published earlier reported the highly specific transmission of the New Jersey variety of potato yellow-dwarf virus (*Marmor vastans* var. *agalliae*) by *Agallia constricta*, but not by the related insect *Aceratagallia sanguinolenta* and the equally specific transmission of the New York variety of potato yellow-dwarf virus (*M. vastans* var. *vulgare*) by the latter of the two leafhoppers but not by the former. Additional experiments have failed to reveal any exception to these results, and strengthen the evidence that the specificity is absolute. The similarities of the symptoms caused by the two viruses in *Nicotiana rustica* and other hosts and protection tests reveal their close relationship. The presence of rusty-brown vein necrosis in *Trifolium incarnatum* infected by the New Jersey variety of the virus and its absence in plants infected by the New York variety have provided a consistent and ready means of distinguishing the two viruses.

*Studies on the Etiology and Physiology of Tumefactions.* BRAUN, ARMIN C., PHILIP R. WHITE, AND THOMAS LASKARIS. 1. Bacteria-free crown-gall tumors. 2. Tumor formation by attenuated crown-gall bacteria in the presence of growth-promoting substances.

*Cottonseed Treatments: Seeding Rates, Yields, and Profits.* CHESTER, K. STARR, AND W. W. RAY. Five years' tests of cottonseed treatments in Oklahoma have demonstrated that: (1) The major organisms producing seedling disease in Oklahoma differ from those of the Southeast: *Rhizoctonia solani* Kühn is dominant and *Glomerella gossypii* (South.) Edg. is negligible; (2) disinfestant seed treatments, while helpful in the Southwest, are less so than acid treatments, which aid escape from *Rhizoctonia* through accelerated seedling development; (3) in order of effectiveness in rhizoctonia control were ethyl mercury phosphate, ethyl mercury iodide, ethyl mercury borate, methyl mercury napththol sulfamide, and tetrachloro-para-benzoquinone; (4) much of the value of seed treatment is lost unless planting rates are reduced from the present extravagant rates to compensate for improved stands due to treatment. Total yields from treated seed planted at 6 lb. per acre, without thinning, approximately equalled those from treated seed planted at 15 lb. per acre, with thinning. Yield increases from treated compared with nontreated seed were much greater at the reduced planting rate (58%) than at the greater rate (20%). Demonstrated advantages of seed treatment in Oklahoma include saving of 60 per cent of seed, elimination of thinning cost, and 19 per cent yield increases, but the maximum profit can be obtained only if seeding rates of treated seed are drastically reduced.

*New Wood-preservative Adaptations for Poles and Crossarms.* COLLEY, REGINALD H. Emergency restrictions on timber and preservatives have accelerated the use of substitute woods and the development of new preservatives. Full scale commercial experiments have demonstrated that mixtures of creosote, petroleum and pentachlorphenol can be used for pressure treatment of southern pine poles, and for open-tank treatment of western cedar poles, and of Douglas fir, jack pine and Norway pine crossarms. Penetration in the above ground sections of cedar poles is facilitated by shaving off the sapwood with special machines until the sapwood depth is less than ½ inch. In the other materials cited complete penetration of the sapwood and some penetration of the heartwood can be obtained readily, insuring very long resistance to attack by wood-destroying fungi. Satisfactory treatments also can be secured for poles by combining full-length treatment with salt preservatives and butt treatment with creosote or creosote solutions. The groundline area of standing poles can be treated with sodium fluoride of borax and creosote to prolong the service life of existing installations.

*Significance of Synergism and Antagonism Between Toxicants.* DIMOND, ALBERT E., JAMES G. HORSFALL, AND J. W. HEUBERGER. Synergism may be conceived as unexpected improvement, antagonism as unexpected loss, in toxicity in a mixture of two materials. Where either phenomenon occurs the slope of the toxicity curve is altered, indicating that a new compound is formed. Synergism in a mixture is denoted by a drop, antagonism by a rise, in LD50 value (quantity for 50 per cent level of response). Intermediate LD50 values are difficult to assess.

They may be assessed by at least 2 methods: one is through use of an organism not affected by one of the components. Another is to vary the proportionality of the components in a series of mixtures, and to assess the LD value for each mixture.

The following two-way systems show synergisms Cuprous oxide + metallic oxides, cuprous oxide + sulphur, metallic oxides + sulphur, sulphur + mercaptobenzothiazole, sulphur + diphenyl amine. Metallic salts other than oxides have shown no synergism. Antagonism is shown by metallic oxides + mercaptobenzothiazole. Three-way systems have shown neither additional synergism nor antagonism over two component systems. The results offer a procedure for stretching fungicide supplies in the war effort.

*Helminthosporium turcicum Leaf Blight of Field Corn Inbreds and Hybrids.* ELLIOTT, CHARLOTTE. During the past two seasons, *Helminthosporium turcicum* has caused heavy leaf spotting, particularly on some of the hybrids being grown in the Corn Belt States and eastward. Some hybrids have been much more susceptible to this leaf spotting than others. In some hybrids the lower leaves have been destroyed; in others all of the leaves to the top of the plant have died prematurely. Records at Beltsville, Maryland, during the past season have shown that some inbred lines, such as Ky. 27, C.I. 23, K4, and Ia. L317, are resistant to spotting by *H. turcicum*, others, such as Ia. ITE 701, C.I. 6, Ind. Tr, and K64, very susceptible, and some such as Ill. Hy, C.I. 187–2, Ind. 38–11, Ia. I205, and Ind. WF9, intermediate. All crosses with the very susceptible inbred Ia. ITE 701 were moderately or heavily infected. The humid wet weather conditions were particularly favorable to development of this widely distributed species during the past season.

*Breeding for Development of a Timber Type of Disease-resistant Chestnut.* GRAVES, ARTHUR HARMOUNT. The Japanese chestnut (*Castanea crenata*) is blight-resistant, but of comparatively low stature. The American chestnut, *C. dentata*, is susceptible to blight but is of tall stature. Hybrids of the 2 species, made in 1931, show that the American species is dominant as regards growth habit, and incompletely dominant as regards blight susceptibility. Further breeding of the $F_1$'s is necessary to obtain the desired combination of tall, erect growth and disease-resistance. Some of the $F_2$'s obtained (now 5 years old) give evidence of the desired growth habit; and, through inoculation tests, of the required disease resistance. Diagrams illustrating this situation, photographic enlargements of these and other crosses, as well as other material, are presented.

*An Undescribed Disease Causing Rapid Dying of Oak Trees.* HENRY, BERCH W., AND C. S. MOSES. A rapid dying of trees in the red oak group has been severe in Wisconsin during the past decade. In 1941 and 1942 a fungus, tentatively placed in the genus *Chalara*, was isolated from the wood of 116 of 122 symptom trees that were sampled but was not obtained from 56 nonsymptom trees. Stem inoculations with 6 isolates of the fungus were successful on 73 of 88 woodlot trees from 1½ to 10 inches d.b.h. Koch's postulates were completed with these isolates. The first symptom of the disease is a slight crinkling of the leaves, that soon become bronze and then brown. The leaves may fall at any symptom stage or remain dried and curled on the tree. Occasionally, dark streaks are found in the current year's wood of branches and stem. Leaf symptoms of the comparatively few trees of the white oak group whence the fungus was isolated are atypical. No trees of the red oak group have been known to recover. The presence of the disease throughout most of the southern half of Wisconsin and at a few locations in Minnesota, Illinois, and Iowa has been confirmed by cultural diagnosis.

*Techniques for Localizing Viruses and Intensification of Disease Symptoms.* HILDEBRAND, E. M. The value of special techniques (shading, pruning, defoliation, girdling) for localizing and manipulating viruses and intensification of disease symptoms have thus far been tested for a relatively small number of diseases both indoors and out, but with considerable promise. The virus causing the "X" or yellow-red disease of peach can be controlled almost at will on several hosts. In experiments on peach seedlings and *Prunus besseyi* diseased peach buds were inserted in several plants in several positions and various devices tested to manipulate virus movement of which shading proved best. By shading the virus can be forced past but not into an unshaded branch, symptoms developing only in the shaded branch during the current season. The position of the diseased bud on the stem was important because it had to be in the path of the food movement mobilized by

the particular technique employed. On single-stemmed peach seedlings the amount of plant shaded and position of diseased bud on the stem can be regulated to determine the minimum effective distance through which the virus can be controlled. Intensification of symptoms is dependent on the degree of succulence attained. Limited studies on other viruses tend to confirm these findings.

*Derivatives of Tomato that Tend to Escape Tobacco-Mosaic Disease.* HOLMES, FRANCIS O. *Lycopersicon chilense* Dunal, the Chilean tomato, was found considerably more difficult to infect with tobacco-mosaic virus than the cultivated tomato, *L. esculentum* Mill. The hybrid *L. esculentum* ♀ × *L. chilense* ♂ proved to be infected much more readily than the Chilean parent and only a little less readily than the cultivated tomato. Nevertheless, a few seedlings in subsequent generations that were derived indirectly from the almost sterile hybrid were about as capable of escaping infection as *L. chilense* itself, thus showing heritability of klendusity. Whether this heritable characteristic can be incorporated in cultivated tomato varieties without interference with quality or yield of fruit remains to be demonstrated.

*Inheritance of Pathogenicity and Certain Mutant Characters in Venturia Inaequalis.* KEITT, G. W., M. H. LANGFORD, AND J. R. SHAY. Only 2 types of pathogenic reaction have been encountered in monoascosporic lines freshly isolated from naturally occurring perithecia (*normal* or nonmutant lines), *lesion* (P) and *fleck* (p). With normal parental lines and selected host varieties, crosses of *lesion* × *lesion* lines uniformly gave 8 lines *lesion; fleck* × *fleck*, 8 lines *fleck; lesion* × *fleck*, 4 lines *lesion*, 4 lines *fleck*. In all adequately studied crosses, factors for *lesion* (P) and *fleck* (p) have segregated 1: 1, behaving as alleles. An isolate may incite lesion reaction on one variety, fleck on another. Cultural mutant *tan* (T) × *normal* (t) gave asci containing 4 lines *tan*, 4 lines *normal*. All lines carrying *tan* were noninfectious. Cultural double mutant *tan-nonconidial* (T Nc) × *normal* (t nc) gave lines of *tan, nonconidial, tan-nonconidial,* and *normal* (nontan-conidial), different combinations occurring in different asci. *Tan* and *nonconidial*, respectively, segregated from their *normal* alleles 1: 1. Lines carrying *nonconidial* without *tan* were noninfectious, except on McIntosh on which they incited flecks instead of the lesion reaction of the line before mutation. Studies of crosses of these mutant lines × normal lines indicate that these mutations have suppressed, but not changed, the factor or factors for pathogenicity.

*Control of Seed-potato Virus Diseases.* KOCH, KARL. Virus diseases affecting seed potatoes have been controlled by a 4-step program of production. The first step in this program is the indexing of several hundred single-stalk hills of potatoes in Florida in the winter months. By this means a small stock of potatoes relatively free from virus diseases is obtained. These potatoes are called the elite seed. In the second step this élite seed is increased by planting it by the tuber-hill-unit method the following spring in an isolated area in Maine. These potatoes are carefully rogued during the growing season and the tubers produced are called the foundation seed. The third step consists of planting this foundation seed by the tuber unit method the following year. Again the field is isolated and rogued carefully. The potatoes produced from the third step are called stock seed. This disease free stock seed is then used the following year in the final step for planting a large acreage of potatoes that will produce tubers in quantity suitable to use for seed by the table-stock producer. Careful checking of this seed by means of Florida tests and by field readings has shown this program to be effective.

*Transmission of Cranberry False Blossom from Tomato to Cranberry Plants by Dodder (Cuscuta campestris).* KUNKEL, L. O. In a former communication transmission of false-blossom virus from cranberry to tomato and other herbaceous plants by dodder was reported. Recently, the virus has been taken from tomato to cranberry by the same parasite. Demonstration that dodder can transmit false blossom to cranberry plants suggests the possibility that it may spread the disease in the bogs.

*A Laboratory-greenhouse Method of Evaluating Fungicides by Means of Tomato-foliage Diseases.* MCCALLAN, S. E. A., AND R. H. WELLMAN. Tomato plants, var. Bonny Best, six inches tall are sprayed by means of a stationary precision "paint spray gun" apparatus at 40 lb. pressure for 30 sec. Plants are placed on a compound turn-table within a hood, and geometrically varying dosages of fungicide applied. With above constants, 0.7 cc. of spray is deposited per sq. decimeter leaf surface. After drying, a given concentration of spore suspension is applied with an atomizer at 20 lb. pressure for 30 sec. The plants are immediately placed in high-humidity infection chambers for 24 hours and then removed to the greenhouse. Early blight (*Alternaria solani*), septoria blight (*S. lycopersici*), and late blight (*Phytophthora infestans*) are employed. Spores of known age of the two former are produced on potato-dextrose agar and those of *Phytophthora*

on potato slices. Necrotic lesions of early and late blights develop within 2 or 3 days, and must be counted before coalescing; Septoria requires ten days. All lesions on 3 compound leaves are recorded; checks give approximately 200 lesions. Results on sprayed plants are converted to percentages of check, plotted on logarithmic-probability paper and the LD95 obtained for comparison. The dosage-response curves are flatter than in laboratory.

*Possible Chemical Similarity of Virus and Fungous Toxins.* STODDARD, E. M., AND GEORGE A. ZENTMYER, JR. The hypothesis is advanced that the virus of "X" disease of peaches and the fungous toxins involved in Dutch elm disease and verticillium wilt are related chemically, since chemotherapeutic experiments on control of these diseases during the past 3 years have given comparable results in a number of cases. Treatment with 8-hydroxyquinoline sulphate has resulted in striking temporary reduction of X disease and of Dutch elm disease—later in both cases the treated trees succumbed. Quinhydrone, hydroquinone and p-nitrophenol are the 3 most effective chemicals used in permanently inactivating the X virus *in vivo* by bud-soaking, and have also been consistently effective in retarding progress of Dutch elm disease. Hydroquinone and 8-hydroxyquinoline sulphate have significantly reduced wilting of verticillium-infected eggplants. Disease progress is thus either temporarily or permanently prevented by introducing the same chemicals into plants in the case of diseases caused by a virus and by fungous toxins. Although these results could be ascribed to similarity of the effect of the therapeutants on the plant reaction, it is more probable that they indicate a chemical similarity between the fungous toxins and the virus.

*Evidence for the Evolution of Phytopathogenic Viruses from the Chondriome.* WOODS, M., AND H. G. DUBUY. Leaf variegations are caused by abnormal plastids that often behave like viruses. Both may cause similar modifications in specific intracellular enzymes and in cell development. Both may cause similar inhibition or destruction of the normal chloroplasts. Variegation-inducing plastids (chondriosomes *sensu* Guilliermond) and viruses are matroclinously inherited. Cytological evidence of limited intercellular migration of variegation-inducing chondriosomes has been obtained, and in two cases the variegations have been graft-transmitted. Chondriosome-controlled variegations can be arranged to form a "spectrum" in which the aberrant plastids progressively become morphologically and physiologically more like typical viruses. We have prepared (DuBuy and Woods, unpublished) from isolated chloroplasts, after removal of lipoids, 10 to 20 per cent of a nucleoprotein, apparently of the d-ribose type (negative Schiff test). From this the nucleic acid was isolated. It gave strong pentose tests, phosphorus tests, and showed maximum U–V absorption around 2600 A. Solubility characteristics were typical of nucleic acid. These data furnish a chemical "common denominator" for the origin of viruses from the chondriome, many viruses consisting of nucleoprotein, the plastids consisting of this and the chromoprotein complex.

*Detecting Pathological Regions and Nonconducting Tissues in Living Pine Trees by Means of Dyes.* YORK, HARLAN H. Naturally and artificially established white, red, and Scotch pine trees were cut off near the surface of the ground and immediately stood in vessels containing aqueous solutions of dyes from 36 to 72 hours. Certain dyes penetrated the living wood up to the tips of the needles. Regions in the stem in which the dye failed to penetrate were found infected with various species of fungi and bacteria. Such lesions were more abundant in weaker than more vigorous trees. These experiments proved to be very useful in studies on the decline of branches and on pruning.

# PEACH WART

EARLE C. BLODGETT

(Accepted for publication May 18, 1942)

## INTRODUCTION

A recently described virus disease of peach is now known in several orchards of the intermountain Pacific Northwest, particularly in Idaho. It has created considerable anxiety among growers, and, because of the grotesque effects on the fruit and the potential seriousness of the disease, has become of great interest to persons working with peach-virus troubles. The purpose of this paper is to describe more in detail the symptoms of peach wart and record the present knowledge of the disease based upon experimental results and observations during the past 5 seasons.

## HISTORICAL

The first peach-wart specimens to come to the writer's attention were seen on a tree in an orchard near Weiser, Idaho, in June, 1938. This particular tree had borne warty peaches for at least two previous seasons. Later in the same summer affected fruits were sent in for diagnosis from Emmett, Idaho. The writer first reported the occurrence of this disease in July, 1939, when it was called "blister" (1). Further observations proved the name wart to be more appropriate, and it commonly is now so referred to in the literature (2, 3, 4, 5, 6, 8, 9).

Diseased trees have been observed in Adams, Washington, Gem, and Canyon Counties, Idaho, and among experimental trees located in Latah County. Although wart was observed first in 1938, there are several growers in Adams and Gem counties who claim having had warty trees for many years. One orchard operator states that he remembers a warty peach tree on his father's farm about 20 years ago. The nature of the symptoms of this disease lends credence to these reports.

In a letter to the writer, E. L. Reeves, Wenatchee, Washington, who is acquainted with peach wart as known in Idaho, reports, "that wart was found in the Yakima, Washington, district in 1940 and in the Wenatchee district in 1941 and probably had been present for at least ten years."[1] Affected fruits have been sent to Moscow by a grower who lives in Payette, Idaho, but whose orchard is across the Snake River in Oregon. At the present time the known distribution is western Idaho, central Washington, and eastern Oregon.

## SYMPTOMS

The very first indications on fruit affected with wart appear shortly after the calyces have fallen. Bleached blisters or raised welts form on the tissue

[1] The writer does not wish to include here details of the observations in Washington, nor to indicate to whom credit should go for first finding and recognizing the disease there. He extends his thanks to Mr. Reeves for reading this manuscript and making helpful suggestions.

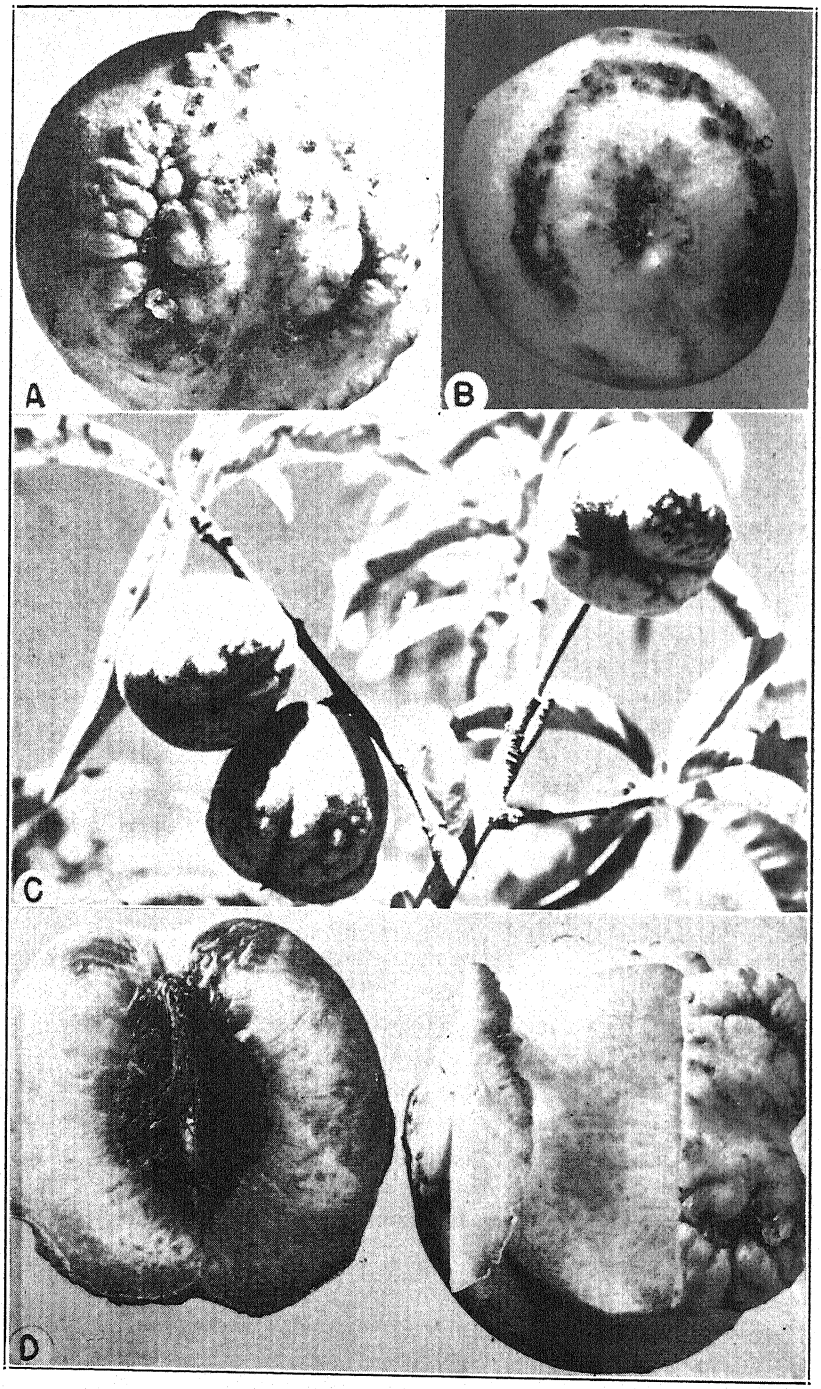



FIG. 1. The peach wart disease. A. Severe, rough, warty type. B. Ring-wart type. C. Warty fruit on tree to which a diseased peach tree had been inarched 15 months before. D. Warty fruit cut to show tissue underneath diseased areas. In all cases warty growths occur predominantly on the stylar ends of fruits.

near the stylar end, but, in severe cases, they later involve half or more of the fruit. The wart tissue enlarges as the fruit grows, and appears more conspicuous as the skin of the peaches becomes less pubescent. In extreme cases, dwarfing and deformity of the fruit are evident; but, generally, slightly affected fruit will attain good size, and the warty condition will look somewhat like a limb rub.

Affected tissue varies in color from a light tan to a red and may be rough with warty outgrowths conspicuously raised (Fig. 1, A, C), or it may appear rather smooth or cracked and russetted (Fig. 2, B). Gumming is usually present and often extremely severe (Fig. 2, F). The warty tissue apparently is quite superficial; and, although the underlying tissue is coarse and filled with gum pockets (Fig. 1, D), there has been no characteristic taste noted. In one collection of specimens from a tree at Caldwell, Idaho, the warty tissue was very hard and bony, much like the pit as it hardens (Fig. 2, C). Usually, however, it is merely tougher than normal tissue but cuts easily. The histological origin of warty tissue has not been determined.

With three exceptions, trees bearing warty fruits gave no other evidence of any abnormality. One tree showed a slightly darker green foliage than normal and two trees, noted first in August, 1941, had a mottled foliage pattern very similar to that of peach mottle (6). Wart-affected trees make normal growth, bear heavily, and appear normal, except for the fruit symptoms. It is of interest to note that at the Mesa orchard, Mesa, Idaho, peach wart, the western X disease, rusty spot (7), and powdery mildew have been seen on the same tree. At one time there appeared to be some possibility that wart and rusty spot might have some connection but now this seems to be unlikely.

As already noted, there are extreme variations in appearance and amount of injury on individual fruits. Certain types of symptoms have given rise to such tentative terms as "crease wart," "beady wart," "smooth wart," and "ring wart."

Primarily, on the basis of observations, but supported by some tests, it appears likely that "crease wart" and "beady wart" are not caused by the wart virus. These symptoms seem to be more often associated with varietal characteristics (crease wart on the Rio Oso Gem) and with possible insect damage (beady wart). The "crease wart" type is represented by small beady warts along the suture. The "beady wart" type (Fig. 2, E) may occur anywhere on the fruit, and usually is associated with what appears to be early feeding damage by some insect. The fruit shown in figure 2, C, exhibits wart along the crease or suture, but this tissue is very hard and bone-like.

The "smooth wart" type was found predominant on one tree but indications are that "smooth wart" (Fig. 2, B, F) is within the range of usual symptoms of peach wart. "Ring wart" has been noted primarily on trees at Mesa, Idaho, and rarely in other orchards. In the case of "ring wart" the warty tissue is arranged in peculiar ring-like patterns (Fig. 1, B, and Fig. 2, A, G). In a budding test involving cions from the Mesa orchard a

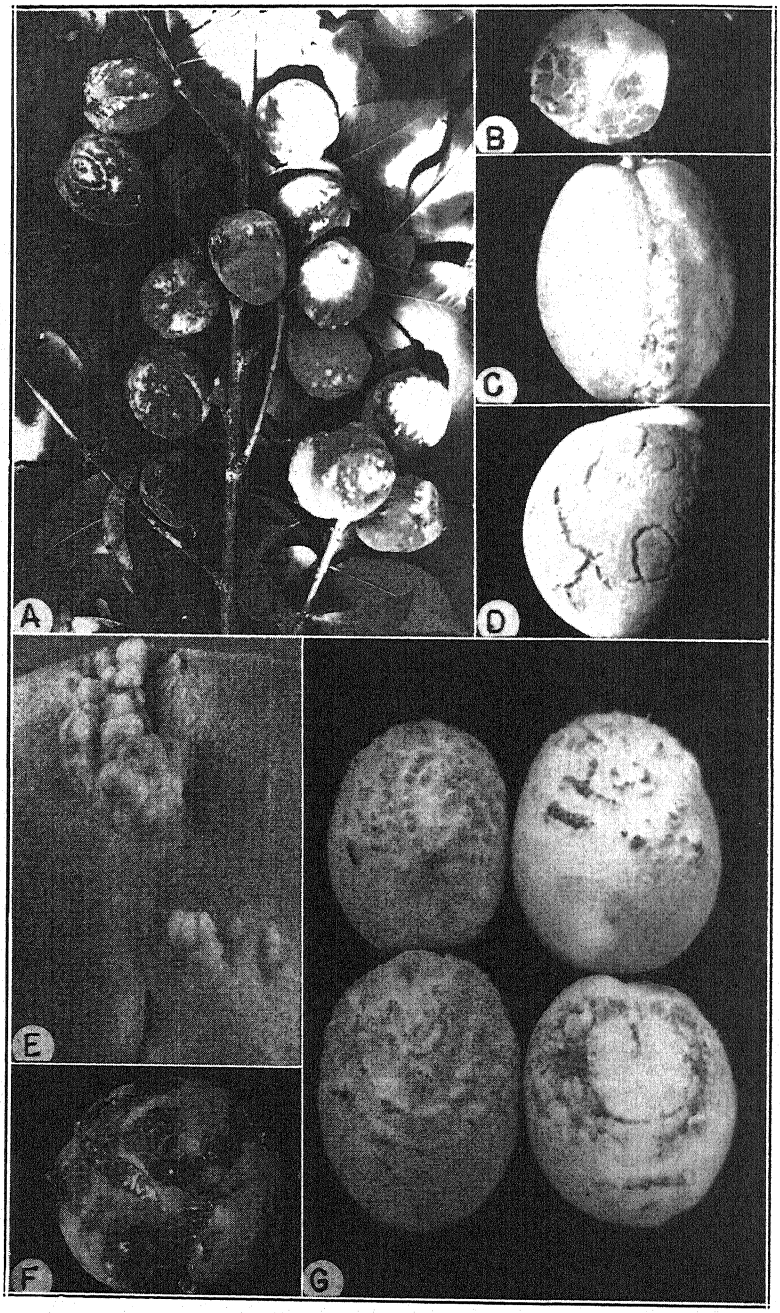



FIG. 2. Peach wart. A. Severe, rough-wart and ring-wart types on Candoka. B. Smooth-wart type. C. Wart growth along the suture but the tissue is hard and bony. D. An interesting variation of ring wart confined to fruit far removed from contact points on the tree to which a warty tree had been inarched. E. Type of wart tissue known as beady wart (probably not caused by the virus). F. Smooth wart showing severe gumming. G. Ring wart occurring naturally in the orchard.

TABLE 1.—*Results of transmission tests by budding and inarching with cion wood and trees affected with peach wart*

| Location | Orchard | Stock variety | Source of buds | No. of buds inserted | Date budded | Condition of inserted buds or grafts | Results on stock |
|---|---|---|---|---|---|---|---|
| 1. Moscow | Greenhouse | Slappey | P–14 J. H. Hale (Weiser) | 8 | March, 1940 | May 9, 1940, all buds dead | Two fruits, but no symptoms |
| | | | | | | April, 1941 | Six fruits, all warty |
| | | | | | | April, 1942 | Six fruits, all warty |
| 2. " | Station | Elberta | P–3 J. H. Hale (Weiser) | 3 | Sept. 22, 1938 | June, 1939, 1 bud alive, 2 buds dead | No fruits produced |
| | | | | | | June, 1940 | " " " |
| | | | | | | June, 1941 | A few peaches, all warty |
| | | | | | | June, 1942 | No fruit |
| 3. Emmett | Modine | Candoka | P–14 J. H. Hale (Weiser) | Whole tree inarched several contacts | Mar. 14, 1940 | June 6, 1940, some contacts united. Diseased tree did not fruit, died Aug. 1940 | Some fruits with suspected symptoms |
| | | | | | | June 12, 1941 | Heavy crop. Fruit very warty and worse on branches contacted |
| | | | | | | June, 1942 | Fair crop, all fruit warty |
| 4. " | " | Elberta | P–16 seedling (Mesa) | " | " | June 6, 1940, some contacts united | (Diseased tree bore heavy crop of warty fruit.) Some peaches with suspected symptoms |
| | | | | | | June 12, 1941 | Heavy crop on previously healthy tree. Fruit very badly affected but worse on branches contacted |
| | | | | | | June, 1942 | Heavy crop, all fruit very warty |
| 5. " | " | " | " | 35 | " | June 6, 1940, 3 buds grew, 17 buds alive, 15 buds dead | Three or 4 fruits with suspected symptoms |
| | | | | | | June 12, 1941, 3 shoots, several buds alive | Heavy crop. Nearly every fruit affected with wart |
| | | | | | | June, 1942 | Fair crop, all fruit very warty |
| 6. " | " | " | P–4 Var.? (Emmett) | 12 | Sept. 5, 1940 | Aug. 1, 1941, 2 buds grew, 6 buds alive, 4 buds dead | Fair crop. No affected fruit was found |
| | | | | | | June, 1942 | Light crop, fruit warty, leaves mottled |

TABLE 1.—(*Continued*)

| Location | Orchard | Stock variety | Source of buds | No. of buds inserted | Date budded | Condition of inserted buds or grafts | Results on stock |
|---|---|---|---|---|---|---|---|
| 7. " | Wagner | J. H. Hale | P–16 seedling (Mesa) | 26 | Mar. 14, 1940 | June 6, 1940, 4 buds grew, 13 buds alive, 9 buds dead | Some fruits with suspected symptoms |
| | | | | | | June 12, 1941 | Fair crop, fruit very warty |
| | | | | | | June, 1942 | Tree removed, fall of 1941 |
| 8. " | " | seedling | " | 6 | " | June 6, 1940, 1 bud grew, 3 buds alive, 2 buds dead | No fruit |
| | | | | | | June 12, 1941 | Fair crop, one fruit on stock very warty |
| | | | | | | June, 1942 | 17 peaches very warty on cions. Some warty, some healthy on stock |
| 9. " | " | " | P–4 Var.? (Emmett) | 9 | Sept. 5, 1940 | June 12, 1941, 1 bud grew, 1 bud alive, 4 buds dead | No fruit |
| | | | | | | June, 1942 | 6 warty fruits (4 warty fruits on cion) |
| 10. " | Thome | Rochester | P–25 E. Crawford (Emmett) | 7 | " | June 12, 1941, 5 buds grew, 2 buds alive | No fruit |
| | | | | | | June, 1942 | Tree completely top-worked (13 warty fruits) |
| 11. Parma | Station | Italian prune | P–16 seedling (Mesa) | 16 | Mar. 12, 1940 | June 19, 1940, 2 buds grew, 14 buds dead | No symptoms noted on foliage or fruit |
| 12. " | " | Same tree as No. 11 | P–25 Crawford (Emmett) | 3 | Sept. 6, 1940 | June 13, 1941, 2 buds grew, 1 bud dead | No symptoms noted on foliage or fruit |
| | | | | | | June, 1942 | One peach fruit on cion normal. No symptoms noted on foliage or fruit |

TABLE 1.—(*Continued*)

| Location | Orchard | Stock variety | Source of buds | No. of buds inserted | Date budded | Condition of inserted buds or grafts | Results on stock |
|---|---|---|---|---|---|---|---|
| 13. " | " | Italian prune | P–16 seedling (Mesa) | 18 | Mar. 12, 1940 | June 10, 1940, all buds dead | No symptoms noted on foliage or fruit |
| 14. " | " | Same tree as No. 13 | P–25 E. Crawford (Emmett) | 9 | Sept. 6, 1940 | June 13, 1941, 5 buds grew<br>3 buds alive<br>1 bud dead | No symptoms noted on tree or fruit |
| | | | | | | June, 1942 | Tree removed, spring, 1942 |
| 15. Caldwell | Saxton | J. H. Hale | P–15 J. H. Hale (Caldwell) | 14 | Mar. 16, 1940 | June 10, 1940, 2 buds grew<br>2 buds alive<br>10 buds dead | Heavy crop but no symptoms noted |
| | | | | | | June 13, 1941 | Heavy crop but no symptoms noted. (Bud wood was not diseased) |
| 16. " | " | Candoka | " | 16 | " | June 10, 1940, 2 buds grew<br>3 buds alive<br>11 buds dead | Heavy crop but no symptoms noted |
| | | | | | | June 13, 1941 | Heavy crop but no symptoms noted. (Bud wood was not diseased) |
| 17. " | Murphy | J. H. Hale | P–11 Halberta (Caldwell) | 10 | Sept. 7, 1940 | June 13, 1941, 4 buds grew<br>3 buds alive<br>3 buds dead | Small crop because of frost but fruit warty. Some leaves mottled. Tree removed, fall, 1941 |
| 18. " | " | Halberta (P–11) (warty) | Healthy J. H. Hale | 10 | Sept. 7, 1940 | June 13, 1941, 2 buds grew<br>4 buds alive<br>4 buds ? | Fruit warty. No fruit on bud shoots |
| | | | | | | June, 1942 | Tree removed, fall, 1941 |

TABLE 2.—*Incidence of peach wart*[a] *during 1939–1941 in part of the peach orchard at Mesa, Idaho*

| Row | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | | 6 | | | 7 | | 8 | | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tree | '39 | '40 | '41 | '39 | '40 | '41 | '39 | '40 | '41 | '39 | '40 | '41 | '39 | '40 | '41 | '39 | '40 | '41 | '40 | '41 | '40 | '41 | '41 |
| 0 | | | | | | | | | | | | | | | | | | | | | | | – |
| 1 | | | | | | | | | | | | | | N | | | | | | | | | w |
| 2 | | | | | | | | | | | | | | W–\|–E | | | | | | | – | – | w |
| 3 | | | | | | | | | | | | | | S | | | | | | | – | – | w |
| 4 | | | | | | | | | | | | | – | – | – | | | | – | – | – | – | – |
| 5 | | | | | | | | | | – | – | – | – | w* | w* | – | w | – | w* | w* | – | – | – |
| 6 | | | | – | – | – | – | – | – | w | w | w | – | – | – | – | – | – | – | – | – | – | w* |
| 7 | – | – | – | – | – | – | – | – | – | w | w | w | – | w | – | – | – | w | – | – | – | – | 0 |
| 8 | – | – | – | – | – | – | w | w | w | w[c] | 0 | 0 | – | – | – | – | w | w | – | w* | – | – | w |
| 9 | – | – | – | – | – | – | w | w | w | w | w | w | – | – | – | – | w | w* | – | – | – | – | – |
| 10 | – | – | – | – | – | – | w | w | w | w | w | w | – | w | w* | – | w | – | – | – | – | – | – |
| 11 | – | – | – | – | – | – | w | w | w | 0 | 0 | 0 | – | w* | w* | – | w* | w* | w* | w | – | – | – |
| 12 | – | – | – | – | – | – | 0 | 0 | 0 | w | w | w | – | – | – | – | – | – | w* | w | – | – | – |
| 13 | – | – | – | – | – | – | w | w | w | 0 | 0 | 0 | – | – | – | – | w | w | – | – | – | – | – |
| 14 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | w |
| 15 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | w* | – | – | – | – | – | – | – | w |
| 16 | | – | – | | – | – | | – | – | | 0 | 0 | | – | w | | – | – | – | – | – | – | – |
| 17 | | – | – | | – | – | | – | – | | – | – | | w | w* | | 0 | 0 | – | – | – | – | w |
| 18 | | – | – | | – | – | | – | – | | – | – | | – | – | | 0 | 0 | 0 | 0 | – | – | w |
| 19 | | – | – | | 0 | 0 | | 0 | 0 | | – | – | | 0 | 0 | | – | – | – | – | 0 | 0 | 0 |

[a] w = wart
– = healthy
* = slight
0 = out

[b] Because of sever frost injury and grasshopper damage, accurate readings were not possible in 1942.

[c] P–16 tree used in inarching and budding tests No. 4, 5, 7, 8, 11, 13, table 1.

slightly different pattern (Fig. 2, D) was shown on fruit growing on adjacent branches or at some distance from the contact points. The fruit on branches brought into contact showed severe, typical symptoms (Fig. 1, C).

### TRANSMISSION STUDIES

A series of transmission tests begun in September, 1938, enlarged and speeded up in 1940 by inarching fruiting trees in the orchard and by greenhouse tests, showed successful transmission of the peach-wart virus by April and June, 1941. The data secured, Table 1, showed that the virus was able to pass from diseased bud wood to the stock (orchard test) and cause warty fruit, in the rather short period from September 7, 1940, to June 13, 1941 (No. 17). One similar case, however, did not effect transmission (No. 6), until the second season.

Good evidence was seen that the greater the amount of diseased bud wood placed on a healthy tree, either a large number of buds or many inarch contacts, the more severe the disease became in one season. Likewise, fruits on the previously healthy tree (inarched) were much more warty on branches in contact with each other than fruits on other branches and farther out on the same branch, (Nos. 3, 4, 5, 17). The virus entered the stock from diseased buds, even if the union between stock and bud tissue was temporary (No. 1). There is good evidence that the disease may be perpetuated in young nursery stock (No. 8).

Tests other than by budding and inarching have not been tried. No root grafts were attempted. The diseased buds were taken from current year's shoots of affected trees, inserted by the shield method and held in place by rubber strips. The two trees to be inarched were dug and transported to the orchard and reset near the bases of the healthy tree and inclined toward them so that several branches and shoots would run approximately parallel. Suitable cuts were made on "stock and cions" and the tissues held together by rubber strips (or wires, in the case of larger branches), then waxed to prevent drying.

### NATURAL INCREASE OF WART IN THE ORCHARDS

Early observations on wart gave no evidence of spread; but, as newer cases came to be known, there appeared to be a slow increase. Several growers reported that when infected trees had been finally removed they discovered that one or more new cases usually appeared later somewhere in the orchard. In one planting near Caldwell, Idaho, five new wart-affected trees appeared in 1941. At Mesa, mappings have been made for 3 years (Table 2). It is now believed that natural spread, although rather slow, does take place. A tree once bearing warty peaches always will, although the severity of symptoms varies and, at first, fruit only on part of the tree or main branches may be affected (Fig. 3). According to the map (Table 2), tree 7, row 5, and tree 5, row 6, showed wart in 1940, but none was noted in 1941. Chance pruning out of infected branches might account for these cases.

HOSTS

The only known host is peach (*Prunus persica* Sieb. and Zucc.) represented by the following varieties: J. H. Hale, Elberta, Candoka, Early Crawford, Halberta, Slappey, July Elberta, and Seedlings. One Quetta nectarine tree has been observed with deformed fruit suggestive of wart but trials are not completed. The budding tests on Italian prune (*Prunus domestica* L.) (Table 1, Nos. 11, 12, 13, 14) have not given definite results. Additional host plants are on trial.

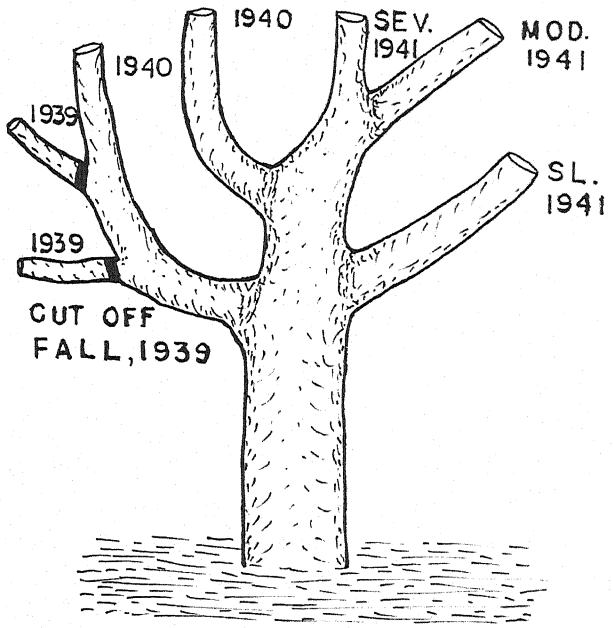



FIG. 3. Peach tree, Wagner Orchard, Emmett, Idaho, affected with wart, showing progress of the virus from branch to branch. Warty fruits were produced on the two branches at the left in 1939. They were cut off in the fall. In 1940 the two large branches at the left showed warty fruit. In 1941 the remaining branches bore warty peaches as indicated.

THE VIRUS

Very little is known regarding the virus causing peach wart. For persons wishing to employ the system of binomial nomenclature the writer, using the suggestions of Francis O. Holmes, proposes the name *Galla verruca* for this virus. Preliminary trials on insect transmission using the leafhopper *Cicadella hieroglyphica* (Say) have been started.

DISCUSSION

So far as the writer is aware no record in the literature on peach troubles describes a disease similar to the one herein referred to as peach wart. The fruits are not just rough and bumpy but show actual wart-like outgrowths or smooth corky areas. The lack of constantly associated leaf and tree symp-

toms makes the grotesque fruit symptoms even more interesting. With so little information regarding the virus itself, the proper classification of the virus and selection of a suitable name are very difficult.

The recognition of one more new virosis of the peach, raises the interesting question of the origin and status of the wart virus. Where did it come from? How long has it been in existence? What are its potentialities? To answer these questions is obviously impossible but they are worthy of consideration in view of recent ideas in regard to strains as pointed out by Kunkel (10). The variation in symptoms (Figs. 1 and 2), and the names "ring wart" and "smooth wart" give some indication of variability in the wart virus. The apparent absence of foliage and tree growth symptoms, the extreme fruit abnormalities, and the lack of similarity to other known peach diseases might lead one to believe that *Galla verrucae* is a "new" virus or at least one whose probable progenitor is unknown.

Peach wart has been known since 1938 and several growers believe that wart has been present in Idaho for over 20 years and there is good evidence of its being present in Washington for at least ten years. These periods are relatively short in regard to the many centuries during which peaches have been grown. The two localities, southwestern Idaho and central Washington, possibly represent separate points of origin. The peach wart disease, however, has been present for comparatively quite a long period in these areas and it seems more plausible to assume that the virus originated here rather than having been introduced. This theory may be discarded if the range of wart is more widespread than known at present. In addition there is the possibility that the disease is caused by a mixed virus infection.

All the early records indicate that wart spreads very slowly in nature and the distribution in orchards is very scattered. This point is of importance in connection with delayed recognition of the disease. A few growers who watch their trees very carefully, and have seen a few new warted trees the last year or so are now much concerned over the potential seriousness. There is some reason to anticipate a sudden widespread increase not unlike that of many of the viruses depending on favorable natural conditions not yet understood. Wart should be regarded as a distinct menace to peach orchards.

## SUMMARY

A virus disease of peach described first in 1939 and reported only from Idaho, Washington, and Oregon is called peach wart. Symptoms are characterized by smooth or rough outgrowths of fruit tissue, particularly near the stylar end. Gumming is usually present and often severe. The virus is readily transmitted by budding and persists in the tree. Peach is the only known host. Natural spread, although slow, takes place. The virus has been tentatively named *Galla verrucae*, although little is known of its character-

istics. Control is by eradication of diseased trees and the use of healthy propagating wood.

IDAHO AGRICULTURAL EXPERIMENT STATION,
MOSCOW, IDAHO.

LITERATURE CITED

1. BLODGETT, E. C. Some obscure peach diseases in Idaho. Pl. Dis. Rptr. 23: 216–218. 1939.
2. ———. Incidence of fruit diseases in Idaho in 1937 and 1938. Pl. Dis. Rptr. 23: 238–245. 1939.
3. ———. Fruit diseases in Idaho, 1939. Pl. Dis. Rptr. 24: 177–182. 1940.
4. ———. Important fruit diseases investigated in "Report of Progress in Solving Idaho's Farm Problems." Forty-seventh Ann. Report for the year ending December 31, 1939. Idaho Agr. Exp. Stat. Bull. 236, pp. 49–51. 1940.
5. ———. Fruit diseases in Idaho, 1940. Pl. Dis. Rptr. 25: 229–235. 1941.
6. ———. Studies on peach viroses in Idaho. (Abstract) Phytopath. 31: 859–860. 1941.
7. ———. Rusty Spot of Peach. Pl. Dis. Rptr. 25: 27–28. 1941.
8. ———. Fruit diseases in Idaho, 1941. Pl. Dis. Rptr. 26: 10–15. 1942.
9. JARDINE, J. T., F. D. FROMME, and H. L. KNIGHT. Rept. on the Agr. Exp. Stations, 1940, p. 109. 1941.
10. KUNKEL, L. O. Genetics of viruses pathogenic to plants. Reprint from Publication No. 12 of the American Association for the Advancement of Science, pp. 22–27. 1940.

# FUSIFORM RUST CONTROL IN FOREST-TREE NURSERIES

BAILEY SLEETH

(Accepted for publication May 8, 1942)

Fusiform rust, *Cronartium fusiforme* (A. and K.) Hedgc. and Hunt, has caused severe losses in southern pine nurseries since 1937, when it was first reported[1] as a nursery disease. Losses of 15 to 35 per cent occurred in certain nurseries in 1938 and 1939. The known loss caused by the disease to slash and loblolly pine seedlings in southern nurseries exceeded 4,000,000 in 1938 and 3,000,000 in 1939. Infection was considerably lighter in 1940 with a loss of approximately 1,000,000 seedlings. Losses in longleaf pine seedlings have been much lower than in either slash or loblolly pine in the same nursery, and usually did not exceed 2 to 3 per cent of the production in any one nursery.

Measures to control fusiform rust were initiated in 1939 at the W. W. Ashe Nursery, Brooklyn, Mississippi, as a part of the Civilian Conservation Corps forest-pathology guidance and service program to facilitate the production of nursery seedlings for reforestation purposes. The control work herein reported was made possible through the cooperation of the Civilian Conservation Corps, the U. S. Forest Service, and the Bureau of Plant Industry. The preliminary work in 1939 yielded inconclusive results; the tests, therefore, were continued in 1940. The measures tried were spraying, oak eradication, late sowing, and chemotherapy. Treatments were confined primarily to slash pine because of its marked importance in southern nurseries, and its pronounced susceptibility to fusiform-rust infection.

Spores (sporidia) of *Cronartium fusiforme* from the alternate host, the oaks, principally the black oaks, infect nursery seedlings from April through June. The period of heaviest infection usually is in April and early May. By early September, slash and loblolly pine seedlings 7 to 8 months old may be found with rust cankers on the stem at or near the cotyledon whorl (Fig. 1, A). The presence of cankers below the cotyledon whorl indicates occurrence of infection directly in the stem, although it is thought that infection commonly occurs through the needles. On longleaf-pine seedlings, canker development appears in the region of the terminal bud and upper part of the taproot, frequently causing a turniplike appearance (Fig. 1, B). Mortality of rust-infected seedlings is usually small in the nursery. However, as pointed out by Lamb and Sleeth,[2] the lethal effect resulting from nursery infection becomes apparent the first year in field plantings where 50–75 per cent mortality may be expected and 100 per cent mortality within a few years.

[1] Lamb, Howard. Rust canker diseases of southern pines. Southern Forest Experiment Station Occasional Paper No. 72. 7 pp. 1937.

[2] Lamb, Howard, and Bailey Sleeth. Distribution and suggested control measures for the southern pine fusiform rust. Southern Forest Experiment Station Occasional Paper No. 91. 5 pp. May, 1940.

SPRAYING

The 1940 spray treatments were divided into 2 general series: a hand-sprayed or plot series in which the effectiveness of different fungicides and spreaders were compared; a power-sprayed or production series involving variations in the spray schedule. The spray schedules were based on the period of active sporidial production. Slash pine, sown March 18, was first sprayed on April 8 and 12, when uredia of *Cronartium fusiforme* appeared

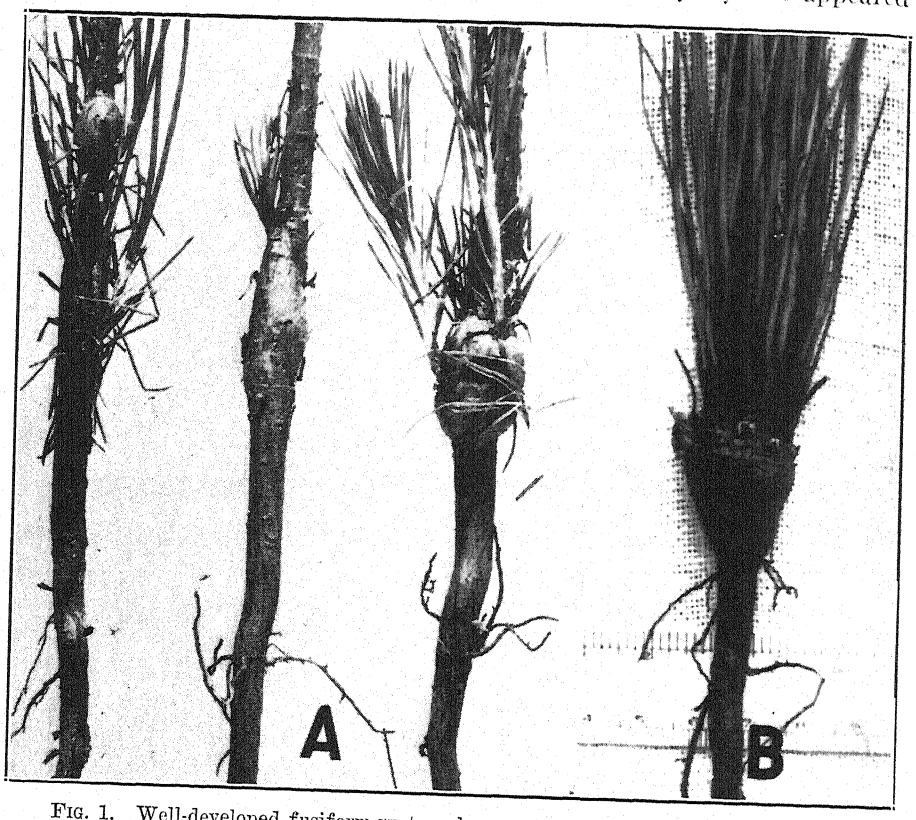



FIG. 1. Well-developed fusiform-rust cankers on 9-month-old pine nursery seedlings. A. Stem cankers on slash pine seedlings. Cankers at or near the cotyledon whorl are common on both slash and loblolly pine seedlings. B. A longleaf pine seedling with rust canker extending into upper part of taproot.

on the oak leaves near the nursery and a few days preceding appearance of telia on the oak leaves. Slash pine, sown April 15, received its first spray application on May 2, after the burlap mulch had been removed and several days after appearance of sporidia. Spraying was discontinued by June 3.

## Spray Materials

Bordeaux mixture, 8–8–100, was the only fungicide applied in the power-sprayed series. In addition to Bordeaux mixture, dry lime-sulphur and Copper Hydro[3] were used in the hand-sprayed series.

[3] Copper Hydro, a commercial fungicide, furnished by the Chipman Chemical Co., Bound Brook, N. J.

In preparing Bordeaux mixture 8 lb. of powdered copper sulphate and 8 lb. of hydrated lime were used in 100 gal. water or in the same ratios when smaller amounts were prepared. Copper Hydro was used at the rate recommended by the manufacturer and dry lime-sulphur at two rates—summer strength and one-half summer strength of liquid lime-sulphur.

### Spreaders and Stickers

Preliminary spraying trials, made in 1939, indicated a considerable difference in effectiveness of spreaders and stickers on slash pine seedlings. In these trials several spreaders and stickers were used and their effectiveness gauged by the amount of visible spray material adhering to the seedlings. The two most promising of these, Santomerse S, and an emulsion of raw linseed oil and fish oil soap, were included in the hand-sprayed series. Because of its availability, casein spreader also was used, although it appeared to be one of the less promising adhesives employed in 1939. A stock supply of linseed-fish-oil-soap emulsion was prepared at the start of the spraying season and used as needed. The stock emulsion, sufficient for 100 gallons of spray mixture, consisted of 3 lb. raw linseed oil and 3 lb. liquid fish-oil soap emulsified by vigorous agitation in 3 pints of water. By doubling these amounts a 6–6–6 emulsion was secured.

### Sprayers

A 3-gal. compressed air sprayer proved more convenient than a power sprayer for the spraying of small plots and where small amounts of different fungicides were applied. The pressure secured in the hand sprayer was variable and probably low. However, good visual coverage was secured.

In the spray schedule tests in which fairly large quantities of Bordeaux mixture were applied, a power sprayer was used. The spray tank was minus a mechanical agitator, and the pressure maintained was comparatively low, fluctuating between 75 and 150 lb. Absence of mechanical agitation and comparatively low pressure no doubt influenced the effectiveness of the spray.

### Plot Arrangement

A block consisting of 5 slash pine nursery beds, sown March 18, was divided into 20 plots for spraying in the hand-sprayed series. Each plot was approximately 100 ft. long by 4 ft. wide. There were 9 treatments and a check distributed at random in each of two sub-blocks.

Two blocks of slash pine, one sown March 18 and the other, April 15, were included in the power-sprayed series. In each block there were 10 nursery beds, each approximately 400 ft. long, divided into 2 sections, an east and a west half. The treatments were arranged systematically in order to facilitate spraying and to cause little or no interference with nursery production. Adequate checks were provided to determine the effectiveness of the treatments. In any case, to be of immediate use and have practical control value,

TABLE 1.—*Reduction of fusiform-rust-cankered slash pine seedlings secured in hand-sprayed plots sown March 18*[a]

| Fungicide | Spreader used and amount per 100 gallons | | Sprayings | | Seedling classes—diameter of stem at base | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | −1/8 inch | | +1/8 inch | | All seedlings | |
| | | | Total | Final | Total | Cankered | Total | Cankered | Total | Cankered |
| | | | *Number* | *Date* | *Number* | *Per cent* | *Number* | *Per cent* | *Number* | *Per cent* |
| Bordeaux mixture | Santomerse S | 1 pint | 12 | 6/3 | 616 | 0.8 | 53 | 1.9 | 669 | 0.9 |
| Bordeaux mixture | Santomerse S | 2 pints | 12 | 6/3 | 686 | 1.5 | 107 | 3.7 | 793 | 1.7 |
| Bordeaux mixture | Casein spreader | 6 pounds | 12 | 6/3 | 771 | 2.2 | 67 | 4.5 | 838 | 2.4 |
| Bordeaux mixture | L & S emulsion[b] | 3–3–3 | 12 | 6/3 | 697 | 1.3 | 92 | 3.3 | 789 | 1.5 |
| Bordeaux mixture | L & S emulsion | 6–6–6 | 12 | 6/3 | 748 | 1.1 | 73 | 4.1 | 821 | 1.3 |
| Copper Hydro | Santomerse S | 1 pint | 12 | 6/3 | 744 | 2.2 | 65 | 6.2 | 809 | 2.5 |
| Dry lime-sulphur, 1–100[c] | Santomerse S | 1 pint | 11 | 5/27 | 709 | 5.6 | 62 | 25.8 | 771 | 7.3 |
| Dry lime-sulphur, 2–100 | Santomerse S | 2 pints | 11 | 5/27 | 647 | 5.9 | 101 | 11.9 | 748 | 6.7 |
| Dry lime-sulphur, 1–100 | Casein spreader | 6 pounds | 10 | 5/27 | 800 | 6.4 | 84 | 20.2 | 884 | 7.7 |
| Unsprayed seedlings | ..... | ..... | ..... | ..... | 805 | 12.4 | 44 | 31.8 | 849 | 13.4 |

[a] Seedlings were sprayed twice a week for 4 weeks commencing April 12 and then changed to a schedule of once a week to June 3.

[b] The L & S emulsion was made by emulsifying 3 lb. of raw linseed oil and 3 lb. of liquid fish-oil soap in 3 pints of water. These amounts were doubled to make a 6–6–6 emulsion.

[c] Dry lime-sulphur was used at summer strength, 2–100, and one-half summer strength, 1–100.

TABLE 2.—*Reduction in fusiform rust cankered slash pine seedlings sprayed with a power sprayer using Bordeaux mixture, 8–8–100 and 1 pint of Santomerse S*[a]

| Sprayings | | | Seedling classes—diameter of stem at base | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | −1/12 inch | | 1/12–1/8 inch | | +1/8 inch | | All seedlings | |
| Frequency | Number | Last date | Total | Cankered | Total | Cankered | Total | Cankered | Total | Cankered |
| | | | *Number* | *Per cent* | *Number* | *Per cent* | *Number* | *Per cent* | *Number* | *Per cent* |
| Sown March 18 | | | | | | | | | | |
| Twice a week | 9 | 5/9 | 367 | 1.6 | 320 | 4.1 | 78 | 11.5 | 765 | 3.7 |
| Twice a week | 12 | 5/21 | 439 | 2.3 | 283 | 4.2 | 20 | 0.0 | 742 | 3.0 |
| Twice a week | 16 | 6/3 | 352 | 2.0 | 341 | 2.9 | 24 | 8.4 | 717 | 2.6 |
| Once a week | 5 | 5/7 | 423 | 5.0 | 325 | 8.0 | 42 | 14.3 | 790 | 6.7 |
| Once a week | 7 | 5/21 | 417 | 5.7 | 303 | 6.6 | 27 | 22.2 | 747 | 6.7 |
| Once a week | 9 | 6/3 | 480 | 4.6 | 365 | 3.1 | 46 | 4.3 | 891 | 4.3 |
| Unsprayed | ... | ..... | 1457 | 8.8 | 838 | 14.4 | 165 | 22.3 | 2460 | 11.5 |
| Sown April 15 | | | | | | | | | | |
| Twice a week | 3 | 5/9 | 252 | 2.8 | 244 | 5.3 | 89 | 7.8 | 585 | 4.8 |
| Twice a week | 6 | 5/21 | 176 | 1.1 | 244 | 4.1 | 84 | 9.5 | 504 | 4.0 |
| Twice a week | 10 | 6/3 | 213 | 1.4 | 249 | 2.8 | 55 | 12.7 | 517 | 3.3 |
| Once a week | 2 | 5/7 | 150 | 2.6 | 245 | 6.1 | 114 | 8.8 | 509 | 5.7 |
| Once a week | 4 | 5/21 | 220 | 3.2 | 269 | 4.8 | 61 | 13.1 | 550 | 5.1 |
| Once a week | 6 | 6/3 | 272 | 1.8 | 164 | 5.8 | 34 | 11.8 | 470 | 3.8 |
| Unsprayed | ... | ..... | 727 | 4.4 | 665 | 10.0 | 197 | 19.8 | 1589 | 8.6 |

[a] Spraying of the slash pine sown March 18 commenced on April 8 and for the April 15 sowing on May 2.

large differences between one or more of the treatments and the checks needed to be demonstrated.

## Method of Sampling

A sample consisted of all seedlings in a 1-foot section across a nursery bed, 4 sq. ft. The samples were taken from areas of fairly uniform seedling development in November and December. When lifted, the seedlings from the hand-sprayed series were separated into 2 groups, one less than $\frac{1}{8}$ in. and the other $\frac{1}{8}$ in. or larger, based on the diameter of stem at or near the root collar. Seedlings in the power-sprayed series were separated into 3 groups, those less than $\frac{1}{12}$ in., those $\frac{1}{12}$ to $\frac{1}{8}$ in., and those $\frac{1}{8}$ in. and larger in diameter. All seedlings in the last class were plantable stock and a considerable number of seedlings in the $\frac{1}{12}$- to $\frac{1}{8}$-in. class would, under good planting conditions, have been acceptable for planting stock.

## Results and Discussion

Effectiveness of the spray treatments was indicated by the reduced number of rust-cankered seedlings observed when examined at lifting time. It was realized, however, that in a given sample the number of cankered seedlings may not have been so great as the total number actually infected because of latent infections that had not yet developed into recognizable cankers. In one planting in southern Mississippi it has been reported[4] that latent nursery infections ranged from 4.0 to 19.0 per cent, and that rust cankers apparently develop more slowly on unthrifty and undersized stock and are more difficult to detect than on large thrifty seedlings. The percentage of cankered seedlings in the plantable class, $\frac{1}{8}$-in. diameter class, was consistently higher than in smaller size classes (Tables 1 and 2). Thus a reduction in amount of cankered seedlings in the plantable class is of more practical importance than in the smaller seedlings, which usually are culled out in grading.

All spray treatments in both the hand- and power-sprayed series (Tables 1 and 2) gave some measure of control. Reduction in number of diseased seedlings ranged from 33 to slightly over 90 per cent. The most effective of the 3 fungicides was Bordeaux mixture, 8–8–100. In the hand-sprayed plots, it was especially effective in reducing the number of cankered seedlings in the plantable class from over 30 per cent in the checks to 4.5 per cent and less. Copper Hydro was slightly less effective and the least effective was dry lime-sulphur. The dry lime-sulphur plots received 1 or 2 fewer sprayings than those sprayed with Bordeaux mixture. However, the difference in effectiveness of these two fungicides was too great to be accounted for by the reduction in number of spray treatments. The difference in control value between Copper Hydro and Bordeaux mixture was not great and may have resulted from excess Santomerse S in the former.

[4] Sleeth, Bailey. Mortality of slash pine seedlings infected by *Cronartium fusiforme*. Southern Forestry Notes No. 35. 2 pp. September, 1940.

The effectiveness of Bordeaux mixture in controlling fusiform-rust infection was further demonstrated in the power-sprayed series in which the percentage of cankered seedlings was reduced from 11.5 per cent in the unsprayed checks to 3 per cent or less in two instances.

Santomerse S and an emulsion of linseed-fish-oil soap were somewhat more effective spreaders than casein spreader in reducing the number of cankered seedlings (Table 1). Of the 3 spreaders used, Santomerse S was considered the most desirable for large-scale operations because of its effectiveness, low cost, and ease of handling. When used at the rate of 1 pt. to 100 gal., Santomerse S gave as good as or slightly better control than when the amount was increased to 2 pt. per 100 gal. The effectiveness of the 3–3–3 linseed-fish-oil-soap emulsion was only slightly increased when the concentration of the emulsion was doubled. There appeared to be no advantage in applying more than the minimum amounts tried. The maximum amount of Santomerse S that can be applied will depend on the sort of mechanical agitation in the spray tank. At two other nurseries where the agitation was exceedingly vigorous, there was a tendency for an excessive sudsy condition to develop when 1 pt. per 100 gal. was used. In these instances satisfactory results were secured by reducing the size of the agitator paddles and cutting down the amount of Santomerse S to ¾ pt. per 100 gal. In no case was mechanical agitation eliminated or reduced to a point where there was a tendency for the spray mixture to settle while mixing or spraying.

There was a lower percentage of cankered seedlings in the hand-sprayed than in the power-sprayed series when the same spray combination, Bordeaux mixture and Santomerse S, and comparable schedules were used. The lower effectiveness of the power outfit probably was due partly to lack of mechanical agitation and perhaps partly to low pressure. A modern high-pressure sprayer would have given higher pressure and probably better control than either the hand sprayer or the power machine employed.

The reduction in cankered seedlings was related to the frequency and number of spray applications (Table 2). In all cases where spraying ceased on the same date or comparable dates there was a lower percentage of cankered seedlings in beds sprayed twice a week than in those sprayed once a week. It is evident that during that part of the season when growth is rapid and sporidia are abundant frequent spraying is necessary to keep the rapidly growing seedlings covered with a fungicide. The data (Tables 1 and 2) indicate that the first 3 or 4 sprayings at the start of the spraying season were more important than the last 3 or 4 applications in reducing rust infections.

The period of sporidial production, particularly the pre-peak and peak periods, rather definitely limits any effective spray schedule to the same period. The earliest sporidial production is in turn limited by the development of oak leaves. In central and southern Mississippi this may occur from April 1 to 15. In some years marked by unusually early spring weather, sporidia may appear in late March. Peak production follows very

shortly after the first sporidia appear and usually continues for 2 to 3 weeks and gradually tapers off into a post-peak period that may extend into midsummer. If pine seedlings are well protected with a fungicide until early June, there should be little or no danger from late infections.

It is not clearly evident from the data in tables 1 and 2 as to which one of the spray schedules used was the more practical. In the hand-sprayed plots 12 sprayings gave excellent control with Bordeaux mixture. It might seem that 12 sprayings would be expensive; yet, it is estimated that this number can be made at a total cost of 15 to 20 cents per thousand seedlings, including cost of labor and materials. The number of sprayings necessary for adequate protection will vary with locality, climatic factors, and amount of sporidia produced. However, the tests indicate that 8 to 12 properly timed sprayings should give satisfactory control. Until we have more information on effectiveness of spray materials, spreaders and spray schedules, 8 sprayings probably is the minimum that should be applied. The seedlings should first be sprayed when uredia of *Cronartium fusiforme* appear on the oak leaves in the vicinity of the nursery or when the first oak leaves are two-thirds to fully developed. Spraying should be done twice a week for 2 to 3 weeks, then once a week until early June.

### OAK ERADICATION

An oak-free zone, 1500 feet wide, was established with Civilian Conservation Corps labor around one compartment at the Ashe Nursery in the spring of 1939, and kept free of oak sprouts the following year. Since successful control of the white pine blister rust, *Cronartium ribicola* Fisch., has been secured in most places by a ribes-free zone of less than 1000 feet around white pine stands, it was anticipated that a 1500-foot zone would give some measure of fusiform-rust control to nursery seedlings.

Slash pine was sown on March 18 and April 15 in the compartment protected by the 1500-foot-wide oak-free zone. Also on the same dates, slash pine was sown in another compartment outside the protected area and within 400–500 feet of a fairly heavy oak growth. The seedlings were examined in late November. The method of sampling and examination was similar to that used in the spraying trials.

No reduction in rust infection was secured, for, in each case where the sowing was on the same date, March 18 and April 15, the percentage of infected seedlings was as high as or slightly higher in the compartment protected by the 1500-foot oak-free zone than in the unprotected area (Table 3). The smaller size and greater density of seedlings in the unprotected compartment may have been a factor in keeping the total percentage of cankered seedlings lower. Regardless of what may have caused the slightly lower percentage of cankered seedlings in the exposed area, it was evident that the 1500-foot oak-free zone was not adequate to protect the seedlings from infection. It is not unlikely that the eradication of the oaks for a distance of 1500 feet does give some protection, but evidently not sufficient to justify

expense of removal where black oaks are abundant a short distance outside of the cleared zone.

Oak eradication, if carried out on a sufficiently large scale, should protect a nursery or planting from rust infection, but it is not known how extensive or intensive should be the eradication of the alternate host, the oaks, in case of *Cronartium fusiforme*. Accurate knowledge of certain factors not yet determined, such as longevity of sporidia, distance viable sporidia can be disseminated by wind, and the special relation between oak concentrations and the amount of infection in pine stands, would aid materially in determining what course to pursue in eliminating oaks as a control measure. To determine the width of zone required to give effective control by gradually extending the limits of the zone is an expensive and not too promising procedure.

### TIME OF SOWING

It has been reported by Lamb and Sleeth[5] "that rust infection in a given nursery is lower in beds of slash pine sown late than in beds of the same species sown earlier." In southern forest-tree nurseries slash pine usually is sown in March. Sowing after the first week in April is considered late, though it may continue until May. To secure more accurate information on relation between late sowing and infection, slash pine was sown at 3 different dates at the Ashe Nursery in the spring of 1940 (Table 3).

The sowing on different dates was combined with the oak eradication. In a section of 9 consecutive 400-foot beds in the compartment protected by a 1500-foot oak-free zone, beds 1, 4, and 7 were sown on March 18, beds 2, 5, and 8 on April 3 and beds 3, 6, and 9 on April 15. Also in a compartment outside the oak-free zone a number of beds for spraying were sown on March 18 and April 15. The amount of infection in unsprayed seedlings in these two sowings is shown in table 3.

The percentage of cankered seedlings was regularly lower in the later sowings. Taking the percentage of cankered seedlings in the earliest sowing in the compartment protected by the 1500-foot oak-free zone as a basis, infection was reduced by a sixth in the April 3 sowing and by two-fifths in that of April 15. In the unprotected compartment the reduction in cankered seedlings in favor of the April 15 sowing over that of March 18 was one-fourth.

In all probability the reduced rust infection in the late-sown slash pine may be accounted for by two factors: sporidial production and unfavorable climatic conditions. In the first instance late-sown slash pine may and frequently does miss the pre-peak and part of the peak period of sporidial production because of late germination. Undetermined fungi are found throughout the telia as they grow older; it is suspected that they are parasitic and are instrumental in shortening the period of sporidial production. Higher temperature and reduced humidity probably inhibit infection as the season advances.

[5] See footnote 2.

TABLE 3.—*Relation of a 1500-foot oak-free zone and the time of sowing to fusiform-rust infection*

| Compartment and date sown | Number of samples | Seedling classes—diameter of stem at base | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | −1/12 inch | | 1/12–1/8 inch | | +1/8 inch | | All seedlings | |
| | | Total | Cankered | Total | Cankered | Total | Cankered | Total | Cankered |
| | | *Number* | *Per cent* | *Number* | *Per cent* | *Number* | *Per cent* | *Number* | *Per cent* |
| Slash pine in a compartment protected by oak eradication, 1500 feet to nearest oaks | | | | | | | | | |
| March 18 | 12 | 549 | 7.3 | 798 | 18.4 | 395 | 26.1 | 1742 | 16.6 |
| April 3 | 12 | 36 | 8.3 | 124 | 9.7 | 387 | 15.8 | 547 | 13.9 |
| April 15 | 12 | 155 | 2.6 | 349 | 12.3 | 376 | 12.5 | 880 | 10.8 |
| Slash pine in a compartment not protected by oak eradication, 400 feet to nearest oaks | | | | | | | | | |
| March 18 | 16 | 1962 | 8.8 | 1138 | 14.6 | 209 | 24.4 | 3309 | 11.8 |
| April 15 | 24 | 1488 | 5.0 | 1318 | 10.9 | 281 | 18.1 | 3087 | 8.7 |
| Loblolly pine in a compartment partly protected by oak eradication, 1000 feet to nearest oaks | | | | | | | | | |
| March 18 | 16 | 157 | 5.1 | 651 | 7.1 | 708 | 8.8 | 1516 | 7.6 |

The value of reduction in number of cankered seedlings in the April 3 and 15 sowing in the protected compartment was more than offset by midsummer losses attributed to *Sclerotium bataticola* Taub. A mortality count made on August 16 revealed a loss of 3 per cent for March 18 sowing, 13 per cent for April 3, and 15 per cent for April 15. Losses such as these and difficulty in growing plantable seedlings do not encourage late sowing as a means of controlling rust infection. However, sowing at as late a date as thrifty, plantable seedlings can be grown is preferable to early sowing in those nurseries subject to recurrent losses from rust infection.

### LOBLOLLY AND LONGLEAF PINE

Because of the interest in relative resistance to infection of slash and loblolly pine seedlings in the nursery, there are included in table 3 data on infection of these two species grown under comparable conditions. Three beds of slash pine in the protected compartment and several beds of loblolly pine in an adjacent compartment were sown on March 18 and grown under comparable conditions, with the exception that the loblolly pine was some 200–500 feet nearer to oaks than was the slash pine. Only 7.6 per cent of the loblolly pine seedlings were cankered, compared to 16.6 per cent of the slash pine in the protected compartment, and 11.8 per cent in an unprotected compartment. Similar cases of higher rust infection in slash than in loblolly pine have been observed in other nurseries.

In years of unusually abundant rust infection, considerable infection may be found in longleaf pine seedlings. Such a condition occurred in 1939 where local spots were found in one nursery to have 10–20 per cent of the seedlings cankered, although the average infection was less than 5 per cent.

The power-spray series for rust control on slash pine was duplicated on longleaf pine seedlings at the Ashe Nursery. In the few samples taken, no evident differences were observed between the sprayed and unsprayed beds.

### CHEMOTHERAPY

The use of chemicals to control cereal rusts experimentally is briefly described by Hart and Allison.[6] They report favorable results with certain chemicals of which the most effective were para- and ortho-toluenesulfonylamide. These two toluene compounds were tried in a small preliminary test on slash pine at the Ashe Nursery in the spring of 1940 in an attempt to control *Cronartium fusiforme* infection of slash pine seedlings.

Two series of $4 \times 4$ ft. plots were established on April 26, one in slash pine beds, sown March 18, and the other sown April 15. The amount of para- and ortho-toluenesulfonylamide used ranged from 1 g. to 4 g. per plot. The chemicals were mixed with sand to secure uniform surface distribution. On December 18, 1940, the seedlings were carefully examined for cankers; the results were inconclusive.

The apparent ineffectiveness of both para- and ortho-toluenesulfonylamide

[6] Hart, Helen, and J. Lewis Allison. Toluene compounds to control plant disease. Phytopath. 29: 978–981. 1939.

may have been due to applying them too late. It seems most likely that the period of severe rust infection had passed by the last of April. Observations made in the spring of 1940 indicated that sporidial production had reached or passed its peak by the last week in April. As a control measure it would seem that the possibility of control would have been much greater had the seedlings absorbed the chemical before infection occurred. On the other hand, if there were a true chemotherapeutic effect, one might reasonably expect an inhibiting or, even, lethal effect on the parasite after infection had occurred.

## SUMMARY

Effective though not complete control of fusiform rust, *Cronartium fusiforme*, was obtained in the nursery by spraying. Of three fungicides applied, Bordeaux mixture, 8–8–100, appeared the most effective, Copper Hydro, slightly less effective, and dry lime-sulphur gave the least control.

Both Santomerse S and an emulsion of raw linseed oil and liquid fish-oil soap gave good and slightly better results as spreaders than casein spreader.

An oak-free zone, 1500 feet wide, failed to show perceptible reduction of rust infection in slash pine seedlings.

Mid-April-sown slash pine showed only $\frac{3}{5}$ as many fusiform-rust cankers as that sown a month earlier.

Less fusiform-rust infection was found in loblolly than in slash pine sown on the same date and grown under comparable conditions. Longleaf pine seedlings proved still more resistant to infection.

As a chemotherapeutic measure, neither para- nor ortho-toluenesulfonylamide, applied to the soil surface at about the time of peak sporidial production, gave any reduction in number of cankered seedlings.

GUAYULE EMERGENCY RUBBER PROJECT,
SALINAS, CALIFORNIA.

# THE CALORIFIC VALUE AND CHEMICAL COMPOSITION OF DECAYED CORDWOOD[1]

M. T. Hilborn and F. H. Steinmetz

(Accepted for publication April 27, 1942)

The chemically complex decay of wood by fungi causes a decrease in the calorific value and oven-dry weight of wood. That is, as decay progresses in a piece of wood, the oven-dry weight and amount of heat derived from the complete combustion of the total decaying material decrease. It should not be surprising, considering the specific differences in wood and fungi and the variations in environment, if different investigators find dissimilar relationships between the change in calorific value and that in oven-dry weight. Lehmann and Scheible (7), in the case of pine wood inoculated with fungi and cultured for a period of 6 months, found that 6 of the 8 fungi employed caused losses in calorific value that were greater than those in wood substance. In wood rotted by the other 2 fungi, they found that the percentage losses in calorific value and the percentage losses in dry material were about the same. Vanin and Esupoff (9) observed that white rots lowered the calorific value per gram of oven-dried wood, while brown rots tended to raise it somewhat, conclusions which are not in agreement with the results of Lehmann and Scheible who found no such differential effect. Hilborn (5), in 3 kinds of wood stored under natural conditions and with numerous fungi present, found slower loss in calorific value than in wood substance. This indicated that during decay some components were removed that either detracted from, or at least did not contribute to, heat value.

Under laboratory conditions in pure cultures, Scheffer (8) studied the effect of *Polyporus versicolor* (L.) Fr. on the calorific value of red-gum sapwood and concluded that the calories remaining in the decaying wood were directly proportional to the amount of wood substance remaining. Hilborn (6) studied the effect of *Fomes fomentarius* (Fr.) Kickx upon the calorific value of white birch wood under laboratory conditions and likewise found that the loss in total calories was directly proportional to the loss in wood substance.

Scheffer (8) and Hilborn (6) were the only two investigators who also made chemical analyses of the wood used in their calorific determinations. However, as neither found any progressive differences in the calories per gram of decaying wood, their data provided no basis for determining the relative influence of the various wood components upon heat value. In view of the differences found by Lehmann and Scheible, it is unfortunate that they did not make chemical determinations on their wood samples.

Two questions arose. The first was whether differences in the ratio of calorie loss to weight loss may sometimes vary directly with differences in

[1] Credit is due the Coe Research Fund, University of Maine, for financial support on this project.

chemical composition; the second was concerned with the frequency of differences in chemical composition in which there were no differences in the ratio of calorie loss to weight loss in decayed wood samples. The samples used by Hilborn in another study (5) seemed to offer opportunity to study the question of the relative calorific value of the various wood components, since it was found that, as specific gravity decreased, the calories per gram of the oven-dried wood also decreased somewhat, indicating a change in chemical composition.

In this experiment, which was begun in 1930, a cord of wood was used consisting of split and unsplit 4-foot lengths of three species, namely, red maple, paper birch, and beech. One half of this cord, consisting of approximately equal numbers of split and unsplit lengths, was piled in the woods and the other half in an open field. In the spring, and again in the fall, from 1930 to 1933, inclusive, representative sticks (4 feet long) of split and unsplit pieces of each species were brought into the laboratory and used for all determinations. The method of preparing the samples for analysis is described by Hilborn (5). After the calorific values were determined in 1934, duplicate portions of these samples were stored in paraffin-sealed glass vials for future use with the hope that at some time chemical analyses could be made. This became possible in 1940.

In order to obtain sufficient material for analysis it was necessary to combine all the samples taken during any one year. Thus the spring and fall samples of 1930, from both the woods and the open field, were combined to form the analyzed 1930 sample. Samples for 1931 and 1932 were derived in the same manner. Sufficient material for analysis was lacking in the 1933 sample. These samples consisted of relatively large wood particles, which had to be reduced in size before a chemical analysis could be made. The material was reduced in a hammer mill and, in the main, following the procedure of Scheffer (8), only that portion of each sample passing a 30-mesh sieve and held on a 100-mesh sieve was used for analysis. The methods of analysis used were those compiled by Bray (1). All reported chemical determinations were made on this material. The material that passed through 100- and 200-mesh sieves, respectively, was removed and stored separately.

The data derived by chemical analysis are presented in tables 1 to 3. It must be remembered that a variety of fungi was responsible for the decay in these samples. Fruiting bodies of several white-rot fungi, *Polyporus hirsutus* (Wulf.) Fr., *P. pargamenus* Fr., *Panus stipticus* Bull., *Stereum purpureum* Pers., *Thelophora* sp., and *Daldinia* sp. were collected during the interval of 1930–34 from the wood in storage. No fruiting bodies of definite brown-rot fungi were found, although brown-rot fungi and white-rot fungi other than those identified may have been present.

The data presented in tables 1 to 3 are partly in agreement with what is known about white rots in general. There was no pronounced increase in alkali solubility, such as characterizes the brown rots (2, 3, 4). There was

TABLE 1.—*Some effects of decay on the chemical composition of red maple wood*

| Kind of component | Amount of component expressed as percentage of wood samples at different storage periods | | | | | | |
|---|---|---|---|---|---|---|---|
| | Original sample | With percentage based upon original weight of oven-dry sound wood[a] | | | With percentage based upon weight of oven-dry decayed sample | | |
| | | Nov. 1930 | Oct. 1931 | Nov. 1932 | Nov. 1930 | Oct. 1931 | Nov. 1932 |
| Cold water soluble | 4.1 | ..... | 1.1 | 1.2 | ..... | 1.5 | 2.2 |
| Hot water soluble | 5.6 | 2.1 | 2.4 | 2.2 | 3.3 | 3.4 | 4.0 |
| Alcohol-benzene soluble | 3.0 | 1.2 | 0.9 | 0.7 | 1.9 | 1.3 | 1.4 |
| Total 1% alkali soluble | 21.6 | 10.7 | 12.8 | 10.4 | 16.7 | 18.1 | 19.2 |
| Cellulose | 58.5 | 35.6 | 41.8 | 31.6 | 55.7 | 58.9 | 58.6 |
| Lignin | 25.9 | 21.2 | 18.2 | 13.7 | 33.1 | 25.7 | 25.3 |
| Total pentosans | 16.9 | 10.8 | 15.3 | 12.3 | 17.8 | 21.5 | 22.7 |
| Pentosans in cellulose (cellulose basis) | 11.9 | 12.5 | 13.0 | 12.3 | 12.5 | 13.0 | 12.3 |
| Pentosans in cellulose (wood basis) | 7.0 | 4.5 | 5.5 | 3.9 | 7.0 | 7.7 | 7.2 |
| Pentosans not in cellulose | 9.9 | 7.6 | 9.8 | 8.4 | 11.8 | 13.8 | 15.5 |

[a] Derived from 3 columns at right.

TABLE 2.—*Some effects of decay on the chemical composition of beech wood*

| Kind of component | Amount of component expressed as percentage of wood samples at different storage periods | | | | | | |
|---|---|---|---|---|---|---|---|
| | Original sample | With percentage based upon original weight of oven-dry sound wood[a] | | | With percentage based upon weight of oven-dry decayed sample | | |
| | | Nov. 1930 | Oct. 1931 | Nov. 1932 | Nov. 1930 | Oct. 1931 | Nov. 1932 |
| Cold water soluble | 2.3 | 0.5 | 1.1 | 0.6 | 0.8 | 1.2 | 1.2 |
| Hot water soluble | 3.2 | 1.9 | 2.7 | 1.5 | 2.9 | 2.9 | 3.0 |
| Alcohol-benzene soluble | 2.3 | 0.2 | 0.3 | 0.5 | 0.3 | 0.3 | 1.1 |
| Total 1% alkali soluble | 17.3 | 11.2 | 16.5 | 9.3 | 16.7 | 17.8 | 19.0 |
| Cellulose | 55.7 | 40.8 | 54.8 | 28.2 | 60.9 | 59.0 | 57.6 |
| Lignin | 23.4 | 14.5 | 23.1 | 12.0 | 21.6 | 24.8 | 24.4 |
| Total pentosans | 16.6 | 17.3 | 23.9 | 13.6 | 25.8 | 25.7 | 27.7 |
| Pentosans in cellulose (cellulose basis) | 15.8 | 16.6 | 16.2 | 14.7 | 16.6 | 16.2 | 14.7 |
| Pentosans in cellulose (wood basis) | 8.8 | 6.8 | 8.9 | 4.2 | 10.1 | 9.6 | 8.5 |
| Pentosans not in cellulose | 7.8 | 10.5 | 15.0 | 9.4 | 15.7 | 16.1 | 19.2 |

[a] Derived from 3 columns at right.

TABLE 3.—*Some effects of decay on the chemical composition of paper birch wood*

| Kind of component | Amount of component expressed as percentage of wood samples at different storage periods | | | | | | |
|---|---|---|---|---|---|---|---|
| | Original sample | With percentage based upon original weight of oven-dry sound wood[a] | | | With percentage based upon weight of oven-dry decayed sample | | |
| | | Nov. 1930 | Oct. 1931 | Nov. 1932 | Nov. 1930 | Oct. 1931 | Nov. 1932 |
| Cold water soluble | 2.1 | 0.9 | 1.8 | 2.4 | 1.7 | 2.8 | 4.8 |
| Hot water soluble | 3.3 | 2.1 | 3.1 | 3.9 | 3.6 | 4.6 | 8.0 |
| Alcohol-benzene soluble | 2.2 | 0.9 | 1.2 | 2.0 | 1.7 | 1.8 | 4.0 |
| Total 1% alkali soluble | 21.9 | 11.0 | 15.2 | 12.7 | 19.0 | 23.7 | 26.0 |
| Cellulose | 62.0 | 34.8 | 38.1 | 28.9 | 60.0 | 59.5 | 58.9 |
| Lignin | 29.5 | 12.2 | 14.1 | 10.2 | 21.1 | 21.9 | 20.9 |
| Total pentosans | 20.8 | 15.6 | 18.2 | 14.2 | 26.8 | 28.4 | 28.9 |
| Pentosans in cellulose (cellulose basis) | 13.9 | 15.1 | 18.8 | 16.3 | 15.1 | 18.8 | 16.3 |
| Pentosans in cellulose (wood basis) | 8.6 | 5.3 | 7.1 | 4.7 | 9.1 | 11.2 | 9.6 |
| Pentosans not in cellulose | 12.2 | 9.1 | 11.0 | 9.5 | 15.7 | 17.2 | 19.3 |

[a] Derived from 3 columns at right.

indicated a preferential selection of the pentosans in cellulose, a characteristic of the brown rots. The principal sources of calories, cellulose and lignin, were depleted generally in direct proportion to the calorific value of the oven-dried wood, as is characteristic of some of the white rots.

The chemical analysis did not explain the differences noted previously between loss in calorific value per gram of oven-dried wood and loss in wood substance, namely, slower loss in calorific value than in wood substance.

A chance determination, based upon material that passed the 200-mesh sieve, yielded an increase in calories per gram. Because of this the writers made a complete series of determinations on the residues that were retained by the 100- and 200-mesh sieves, respectively. The data are presented in table 4, and indicate that particle-size resulting from mechanical screening influenced calorific value. In all samples the material that passed the 100- and 200-mesh sieves was progressively higher in calorific value than that retained and used for chemical analysis. As decay progressed in the samples, the calories per gram of oven-dried wood of these particles increased, in general. No appreciable differences were observed in the relative amounts of material which passed through the 100- and 200-mesh sieves in any of the samples, regardless of the amount of decay. Hilborn (5) concluded that the paper-birch wood had lost more heat value through decay than the other two species. This conclusion is apparently borne out by the data in table 4. However, contrary to the earlier results, here there was little or slight change

TABLE 4.—*Calorific value of screened samples*

| Sample | Calories per gram oven-dry | | | Increase in calories per oven-dry gram of sieved samples over analysis sample | |
|---|---|---|---|---|---|
| | Analysis sample | Passing 100-mesh but not 200-mesh | Passing 200-mesh | Passing 100-mesh but not 200-mesh | Passing 200-mesh |
| Red maple | | | | | |
| Original | 4693 | 4742 | 4787 | 49 | 94 |
| 1930 | 4381 | 4407 | 4436 | 26 | 55 |
| 1931 | 4374 | 4839 | 4974 | 465 | 600 |
| 1932 | 4243 | 4509 | 4637 | 266 | 394 |
| Beech | | | | | |
| Original | 4925 | 5093 | 5112 | 168 | 187 |
| 1930 | 4721 | 4782 | 4935 | 61 | 214 |
| 1931 | 4301 | 4611 | 4923 | 310 | 622 |
| 1932 | 4291 | 4518 | 4735 | 227 | 444 |
| White birch | | | | | |
| Original | 4630 | 4721 | 4936 | 91 | 306 |
| 1930 | 4372 | 4402 | 4583 | 30 | 211 |
| 1931 | 4322 | 4523 | 4711 | 201 | 389 |
| 1932 | 4263 | 5054 | 5233 | 791 | 970 |

in calories per gram of oven-dried wood as decay progressed. There was a consistent loss in calories as decay progressed in the samples used for the chemical analysis, but the total loss after 3 years' decay is rather small. The material of smaller particle size showed a heterogeneity with no consistent trend in calorific value as decay progressed.

## SUMMARY

The effect of decay on chemical composition and calorific value was measured in cordwood of three species, red maple, paper birch, and beech. As causes of decay, several white-rot but no brown-rot fungi were known to be present. Although determinations of the calories per gram had shown that the loss in calorific value was slower than the loss in wood substance, indicating a change in chemical composition during decay, chemical analysis failed to demonstrate a correlation between reduction in calorific value and reduction in the amount of any chemical component.

In these chemical analyses two characteristics of the white rots were found, (1) no correlation between alkali solubility and the loss of any other component, and (2) a depletion of both cellulose and lignin proportional to the loss of wood substance. In addition one characteristic of the brown rots was found—the preferential selection of the pentosans in cellulose.

Particle size resulting from mechanical screening in the reduced sample was observed to influence calorific value. Very small particles were higher in calorific value.

AGRICULTURAL EXPERIMENT STATION OF THE UNIVERSITY OF MAINE,
AND THE COLLEGE OF AGRICULTURE, ORONO, MAINE.

LITERATURE CITED

1. BRAY, M. W. Chemical analysis of pulps and pulp woods. Methods used at the Forest Products Laboratory. Paper Trade Jour. Tech. Assoc. (**Sec. Dec. 20**): 59–68. 1928.
2. CAMPBELL, W. G. The chemistry of the white rots of wood. I. The effect on wood substance of *Polystictus versicolor* (L.) Fr. Biochem. Jour. 24: 1235–1243. 1930.
3. ————. The chemistry of the white rots of wood. II. The effect on wood substance of *Armillaria mellea* (Vahl.) Fr., *Polyporus hispidus* (Bull.) Fr., and *Stereum hirsutum* Fr. Biochem. Jour. 25: 2023–2027. 1931.
4. ————. The chemistry of the white rots of wood. III. The effect on wood substance of *Ganoderma applanatum* (Pers.) Pat., *Fomes fomentarius* (L.) Fr., *Poyyporus adustus* (Willd.) Fr., *Pleurotus ostreatus* (Jacq.) Fr., and *Polystictus abietinus* (Dicks) Fr. Biochem. Jour. 26: 1829–1838. 1932.
5. HILBORN, M. T. The calorific value of decayed cordwood. Phytopath. 26: 905–914. 1936.
6. ————. The biology of *Fomes fomentarius*. Maine Agr. Exp. Stat. Bull. 409. 1942.
7. LEHMANN, K. B., and E. SCHEIBLE. Quantitativ Untersuchung über Holzzerstörung durch Pilze. Arch. f. Hyg. 92: 89–108. 1923.
8. SCHEFFER, T. C. Progressive effects of *Polyporus versicolor* on the physical and chemical properties of red gum sapwood. U. S. Dept. Agr. Tech. Bull. 527. 1936.
9. VANIN, S. I., and M. I. ESUPOFF (M. I. EZUPOV). Ueber den Heizwert des faulen Holzes. Trudy Lesnomu Opytn. Delu, (Leningrad) (Mitt. Forstl. Versuchw.) 2: 71–84. 1930. (In Russian. German Summary pp. 83–84.)

# THE EFFECT OF COTTON SEED DUSTING ON EMERGENCE OF SEEDLINGS IN SOIL INFESTED WITH RHIZOCTONIA[1]

W. WINFIELD RAY

(Accepted for publication May 25, 1942)

From the many tests conducted by various investigators working under the auspices of the Cotton Disease Council it has been definitely established that cotton-seedling infections caused by seed-borne organisms can be controlled effectively by seed treatment with either 2 per cent Ceresan (ethyl mercury chloride) or New Improved Ceresan (5 per cent ethyl mercury phosphate). Among the several organisms effectively controlled by the Ceresan treatment, is *Glomerella gossypii* (South.) Edg., which, according to Weindling *et al.* (8), is responsible for the greatest amount of cotton-seedling loss of all the seed-borne organisms.

It has been observed that seedlings produced from treated seed frequently are attacked, before or after emergence, by various soil-inhabiting fungi. In many instances the organism associated with such attacks is *Rhizoctonia solani* Kühn (*Corticium vagum* B. and C.). Some investigators have suggested that *Rhizoctonia* might be controlled, at least partly, by seed treatment. Lehman (2) demonstrated that the germination of Ceresan-treated seed, planted in Rhizoctonia-infested soil, was significantly greater than that of the nondusted seed. But he also observed that plants from dusted seed did not survive post-emergence damping-off to a significantly greater degree than did those from nondusted seed.

The seedling surveys of Miller (3) and Miller and Weindling (4, 5, 6) and those of Ray and McLaughlin (7) have demonstrated that the anthracnose pathogen, *Glomerella gossypii,* is of little consequence as a cotton-seedling pathogen in Oklahoma. Next to some of the species of *Fusarium,* notably *F. moniliforme* Sheld., *Rhizoctonia solani* is the organism most frequently isolated from diseased seedlings. As pointed out by Ray and McLaughlin (7), *Rhizoctonia,* because of its ubiquity and marked pathogenicity, is considered the most important cause of seedling disease in Oklahoma.

The real importance of *Rhizoctonia* may not be revealed in the actual percentage of isolations from diseased seedlings under all conditions. Experiments in our greenhouse and those made by Lehman (2) have demonstrated that seedlings cannot be protected against infection by *Rhizoctonia* by either 2 per cent Ceresan or New Improved Ceresan. The writer has observed that treated seed planted in soil heavily infested with *Rhizoctonia* often do not emerge at all; and, so, on this basis the long skips in rows commonly seen in cotton fields conceivably may be due largely to a high inoculum potential of *Rhizoctonia* in the soil.

[1] Published with the approval of the Director of the Oklahoma Agricultural Experiment Station.

The purpose of the investigations here described was to determine under controlled conditions, insofar as possible, the effectiveness of seed treatment with several fungicidal dusts in the control of pre- and post-emergence damping-off of cotton seedlings by *Rhizoctonia*.

## MATERIALS AND METHODS

The experiments were conducted in the greenhouse where the temperature was maintained between 80° and 100° F. The soil, consisting of 2 parts loam, 1 part sand, and 1 part sewer sludge, had been steam-sterilized about 1 month before it was placed in clean flats and treated with a concentrated formaldehyde solution, as suggested by Guterman and Massey (1) for the control of damping-off. The organism employed in these experiments was isolated from a cotton seedling, and was selected because of its high degree of pathogenicity as determined by infection tests. The fungus was grown 10 days in flasks on a steam-sterilized bran medium, consisting of 1 part bran and 1.2 parts of a 1 per cent dextrose solution (by weight). One week after the soil received the formaldehyde treatment, 75 g. of the bran culture was stirred into each flat of soil (about 1 part of the bran culture to 55 parts of soil by volume). The soil-inoculum mixture was then kept moist for 7 days prior to the planting of the seed.

Deltapine-12 cotton seed from the 1940 crop in Mississippi was delinted in concentrated sulphuric acid, and only the heavy seed derived from the flotation-gravity process was used. Such seed has been shown by numerous tests in the laboratory and greenhouse to germinate 98–100 per cent. Each flat was planted with 160 treated seeds in 8 rows, while the third row from each end of the flat was planted with 20 nondusted seeds. Usually, each experiment consisted of 4 flats and 4 lots of seed each treated with a given chemical dust. The different lots of seed were rotated so that each lot produced a crop of seedlings successively in each of the 4 flats, thus resulting in 4 replications in each experiment. All dusts were applied to the seed at the rate of 3 g. of dust per kg. of seed, except Spergonex, which was applied at double that rate.

Counts were made of the total number of seedlings emerged and those that survived post-emergence damping-off for one week thereafter. The results obtained in each experiment were analyzed statistically by the analysis-of-variance method.

## RESULTS

In all, 7 sets of experiments were made and the results of each were analyzed statistically. The data obtained for total emergence of all seedlings, regardless of the fact that many of those included in the counts later died, are presented in table 1. The underscored numbers indicate statistical significance at 1 per cent over the untreated checks in each experiment. Except for one experiment, in which red copper oxide was statistically superior to the check, the only chemicals giving emergences consistently

superior to the checks were New Improved Ceresan (5 per cent ethyl mercury phosphate), Spergon (tetrachloro-para-benzoquinone), DuBay 740–A (5 per cent ethyl mercury borate), DuBay 1155–HH (5 per cent ethyl mercury iodide) and DuBay 1228–R (methyl mercury naphthol sulfamide). Although Spergon-treated seed gave higher emergence counts than Ceresan-treated seed in every case where both were used in the same experiment, no significant difference existed between them. Since the various experiments were conducted on different dates, the average percentage of germination for

TABLE 1.—*Average percentage of emergence of treated and untreated cotton seed in soil infested with Rhizoctonia*[a]

| Seed protectant | *Number of experiment* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Aver. |
| Check | 22.0 | 11.5 | 5.7 | 5.4 | 24.0 | 24.5 | 10.0 | 17.1 |
| New Improved Ceresan | *50.0* | *86.0* | *68.5* | *65.7* | *85.0* | ..... | *57.6* | 68.8 |
| Spergon | ..... | *88.0* | *83.4* | *69.0* | ..... | *73.7* | *60.1* | 74.8 |
| DuBay 1155–HH | ..... | *73.0* | ..... | ..... | ..... | ..... | ..... | 73.0 |
| DuBay 740–A | ..... | *74.0* | ..... | ..... | ..... | ..... | ..... | 74.0 |
| DuBay 1228–R | ..... | ..... | ..... | *44.0* | *58.0* | ..... | *48.4* | 50.1 |
| Spergonex | ..... | 19.0 | ..... | ..... | ..... | 23.0 | ..... | 21.0 |
| U. S. Rubber 335 | ..... | ..... | ..... | 8.0 | ..... | ..... | 7.9 | 8.0 |
| Sanoseed A | ..... | 25.0 | ..... | ..... | 18.0 | ..... | ..... | 21.0 |
| Cyanamid 154–6–B | ..... | 18.0 | 13.0 | ..... | ..... | 14.6 | ..... | 15.2 |
| Red copper oxide | *48.3* | ..... | ..... | ..... | 37.0 | ..... | ..... | 42.6 |
| Yellow copper oxide | 29.2 | ..... | ..... | ..... | ..... | ..... | ..... | 29.2 |
| Vasco 4 | 30.0 | ..... | ..... | ..... | ..... | ..... | ..... | 30.0 |
| U. S. Rubber 601 | ..... | ..... | ..... | ..... | ..... | 26.4 | ..... | 26.4 |

[a] Each figure based on 4 replications; numbers in italic are highly significant statistically.

seed treated with any particular chemical in one experiment might not be indicative of the true value of the treatment when compared with average percentage of germination for the same chemical in another experiment. In

TABLE 2.—*Emergence ratio of treated to untreated cotton seed in soil infested with Rhizoctonia*[a]

| Seed protectant | Number of experiment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Aver. |
| Check | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| New Improved Ceresan | 2.2 | 7.3 | 11.9 | 12.1 | 3.4 | ..... | 5.7 | 7.1 |
| Spergon | ..... | 7.5 | 14.5 | 12.7 | ..... | 2.9 | 6.0 | 8.7 |
| DuBay 1155–HH | ..... | 6.2 | ..... | ..... | ..... | ..... | ..... | 6.2 |
| DuBay 740–A | ..... | 6.3 | ..... | ..... | ..... | ..... | ..... | 6.3 |
| DuBay 1228–R | ..... | ..... | ..... | 8.1 | 2.3 | ..... | 4.8 | 5.1 |
| Spergonex | ..... | 1.6 | ..... | ..... | ..... | 0.9 | ..... | 1.2 |
| U. S. Rubber 335 | ..... | ..... | ..... | 1.4 | ..... | ..... | 0.7 | 1.1 |
| Sanoseed A | ..... | 2.1 | ..... | ..... | 0.7 | ..... | ..... | 1.4 |
| Cyanamid 154–6–B | ..... | 1.5 | 2.2 | ..... | ..... | 0.5 | ..... | 1.4 |
| Red copper oxide | 2.1 | ..... | ..... | ..... | 1.5 | ..... | ..... | 1.8 |
| Yellow copper oxide | 1.3 | ..... | ..... | ..... | ..... | ..... | ..... | 1.3 |
| Vasco 4 | 1.3 | ..... | ..... | ..... | ..... | ..... | ..... | 1.3 |
| U. S. Rubber 601 | ..... | ..... | ..... | ..... | ..... | 1.0 | ..... | 1.0 |

[a] Each figure based on 4 replications.

table 2, therefore, are presented the ratios between the percentage of germination of the check and each of the chemicals used in all experiments. In each case the emergence of the check is taken as 1.

The average number of plants still surviving and free from infection, 1 week after emergence, in 5 of the experiments, is tabulated in table 3. In no instance, in any of the experiments, was the survival of the seedlings from treated seed significantly greater than that from the nontreated. These results are in accord with those of Lehman (2).

TABLE 3.—*Average number of emerged plants surviving damping-off by Rhizoctonia on the basis of 4 replications of 160 seeds each*

| Seed protectant | Number of experiment | | | | |
|---|---|---|---|---|---|
| | 3 | 4 | 5 | 6 | 7 |
| Check | 0.0 | 0.5 | 4.0 | 2.2 | 4.5 |
| New Improved Ceresan | 10.0 | 7.0 | 15.0 | ..... | 5.5 |
| Spergon | 18.0 | 11.0 | ..... | 4.0 | 5.6 |
| DuBay 1128–R | ..... | 1.2 | 7.0 | ..... | 3.5 |
| Spergonex | ..... | ..... | ..... | 2.5 | ..... |
| U. S. Rubber 335 | ..... | 1.0 | ..... | ..... | 0.5 |
| Sanoseed A | ..... | ..... | 1.5 | ..... | ..... |
| Cyanamid 154–6–B | 0.8 | ..... | ..... | 0.7 | ..... |
| Red copper oxide | ..... | ..... | 7.2 | ..... | ..... |
| U. S. Rubber 601 | ..... | ..... | ..... | 2.7 | ..... |

## DISCUSSION

Certain chemicals, such as DuBay 740–A and DuBay 1155–HH, although showing promise as seed protectants in the regional field tests[2] and in our greenhouse experiments, are not generally available to the public because of manufacturing difficulties. These two fungicides, therefore, were not thoroughly tested. DuBay 1228–R has given stands significantly better than those from the nontreated seed in both these and above-mentioned field tests. It is not now manufactured in quantities. American Cyanamid 154–6–B (an organic mercury dust) has shown exceptional promise as a seed protectant in Arndt's regional tests, but in our Rhizoctonia tests, emergence was not significantly better than that from the nontreaed seed. Sanoseed A (ethanol mercury chloride) has not been effective against attacks by *Rhizoctonia* in these experiments, whereas, in Arndt's regional field tests it has given somewhat erratic results.

The United States Rubber Company's product, Spergon, has in every experiment given emergence counts highly significant over those of the check, and it compares favorably with New Improved Ceresan as a seed protectant. Spergonex, No. 335, and No. 601, all organic compounds lacking heavy metals, and manufactured by the United States Rubber Company, gave emergences that were not statistically significant over those of the nontreated seed. In the regional tests, Spergonex has proved more effective than the

[2] Arndt, C. H. A summary of six years of experimental studies of cotton seed treatment by the Cotton Seedling Committee. Mimeographed report issued at the meetings of the American Association for the Advancement of Science in Dallas, Texas, in 1941.

other 3 products of the United States Rubber Company, and it has compared favorably with New Improved Ceresan. It proved but slightly more effective than Spergon, according to Arndt.[2] The cuprous oxides (red and yellow) and Vasco 4 (zinc oxide) did not prove effective against *Rhizoctonia* in these tests.

SUMMARY

Cotton seed, treated with various fungicidal dusts, and nontreated seed were planted in soil abundantly infested with *Rhizoctonia solani*. Emergence counts made in 7 experiments and survival counts made in 5 were analyzed statistically.

The chemicals regularly giving emergences significantly greater than those of the nontreated were New Improved Ceresan (ethyl mercury phosphate), DuBay 1155–HH (ethyl mercury iodide), DuBay 740–A (ethyl mercury borate), DuBay 1228–R (methyl mercury naphthol sulfamide) and Spergon (tetrachloro-para-benzoquinone).

Although certain chemicals used in seed treatment significantly increased the emergence above that of the nontreated seed, the differences in subsequent survival of plants from treated and nontreated seed were not statistically significant.

The results of these experiments indicate that seed treatment is not an effective means of controlling post-emergence damping-off of cotton seedlings by *Rhizoctonia* when the soil is heavily infested with this fungus.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY,
OKLAHOMA AGRICULTURAL EXPERIMENT STATION,
STILLWATER, OKLAHOMA.

LITERATURE CITED

1. GUTERMAN, C. E. F., and L. M. MASSEY. An improved formaldehyde treatment for damping-off control. Flor. Exch. and Hort. Trade World 84: 11. 1935.
2. LEHMAN, S. G. Cotton seed dusting in relation to control of seedling infection by *Rhizoctonia* in the soil. Phytopath. 30: 847–853. 1940.
3. MILLER, P. R. A survey of cotton seedling diseases and the fungi associated with them. Pl. Dis. Rptr. 22: 260–263. 1938.
4. ————, and R. WEINDLING. A survey of cotton seedling diseases in 1939 and the fungi associated with them. Pl. Dis. Rptr. 23: 210–214. 1939.
5. ————, and ————. A survey of cotton seedling diseases in 1940 and the fungi associated with them. Pl. Dis. Rptr. 24: 260–263. 1940.
6. ————, and ————. A survey of cotton seedling diseases in 1941 and the fungi associated with them. Pl. Dis. Rptr. 25: 378–380. 1941.
7. RAY, W. W., and J. H. MCLAUGHLIN. Isolation and infection tests with seed- and soil-borne cotton pathogens. Phytopath. 32: 233–238. 1942.
8. WEINDLING, R., P R. MILLER, and A. J. ULLSTRUP. Fungi associated with diseases of cotton seedlings and bolls, with special consideration of *Glomerella gossypii*. Phytopath. 31: 158–167. 1941.

# SULPHUR AND COPPER SPRAYS IN RELATION TO APPLE-TREE GROWTH AND YIELD[1]

H. W. THURSTON, JR., AND H. N. WORTHLEY

(Accepted for publication May 5, 1942)

Lime sulphur-lead aresenate sprays, as applied to apples, have long been recognized as causing various types of foliage injury in varying degree, depending upon season, variety, strength of spraying solution, region under consideration, etc. In spite of such injurious effects, lime sulphur is still the standard early-season fungicide wherever scab is a major problem. The possible cumulative effect of lime sulphur on tree growth and yield over a period of years presents a somewhat different problem. Folsom,[2] in 1933, reviewed the published evidence up to that date, and came to the conclusion that in spite of a strong belief in the importance of spray injury in affecting yield and growth "there is still not much evidence altogether of an accurate nature." Folsom's further conclusions from his own experiments in Maine were that "in general, the tendency was for unmodified lime sulphur to increase yield more than to decrease it, and to have no effect upon tree growth." And further, "that the effect of lime sulphur spray injury upon growth and yield may be much less than is commonly presumed."

A further report by Folsom[3] in 1939 indicates a 50 per cent reduction in yield of McIntosh produced by lime sulphur as compared to sulphur spray or dust, over the period 1933 to 1938 inclusive, with but little difference in tree growth.

Christopher[4] recently has reported better tree growth for young trees sprayed with lime sulphur 1–100 and trees receiving sulphur dust than for trees receiving lime sulphur 1–50. He does not report on tree measurements at the start of his experiment and made only one set of measurements, which seem inconclusive.

The experiments here recorded were conducted on young Stayman trees. These trees were set in 1929 in two blocks of 54 trees each, the blocks being part of a larger orchard and separated by several rows of McIntosh trees of the same age. During the first 5 years of their growth these trees were not sprayed, except for a single uniform application of lead arsenate each year to keep down damage from leaf-eating insects. In 1934 the trees all received a single application of sulphur and lead arsenate at petal fall. In 1935, 2 uniform applications were made, at pink and petal fall. In 1936, 6 trees in each block were set apart to serve as checks. Of the remaining trees, half were sprayed with a standard lime sulphur-lead arsenate schedule and half

[1] Authorized for publication on May 1, 1942, as Paper No. 1099 in the Journal series of the Pennsylvania Agricultural Experiment Station. Contribution from the Department of Botany No. 133.

[2] Folsom, D. Apple spraying and dusting experiments—1928–1932—in relation to scab, yield and tree growth. Maine Agr. Exp. Sta. Bull. 368. 1933.

[3] Folsom, D. Yield reduction by lime sulphur on apple trees. (Abstract) Phytopath. 39: 6. 1939.

[4] Christopher, E. P. Influence of sulphur sprays on trunk diameter of young apple trees. Proc. Amer. Soc. Hort. Sci. 39: 8–10. 1941.

with copper phosphate-lime bentonite-lead arsenate mixture (4–8–4–3–100). This program has been continued through 1941, except that in 1940 and 1941 copper zeolite ("ZO") was substituted for the copper phosphate. Trunk

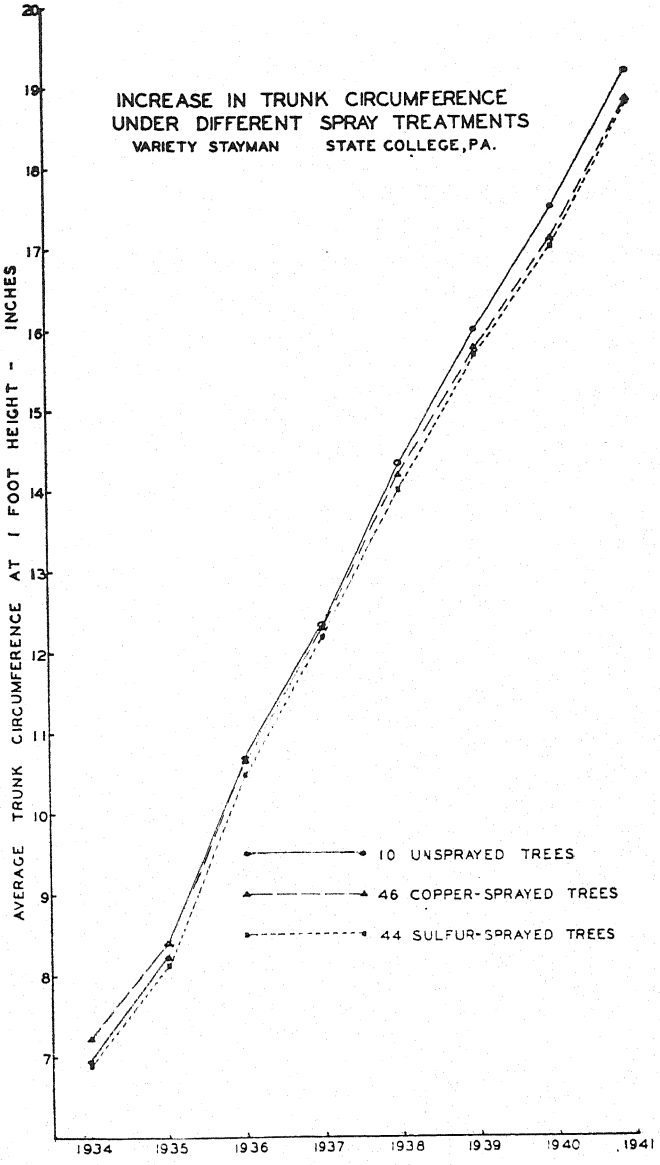



FIGURE 1.

circumferences of all trees have been recorded, beginning with 1934, resulting in a record for 2 years during which the trees were sprayed uniformly and for 6 years during which a spraying differential of lime sulphur vs. copper vs. check was maintained.

For the first 4 years of the differential spraying treatment (1936–1939)

records were kept of the numbers of growing points (buds) and numbers of flower clusters, produced on 3 selected branches from each tree. It was thus hoped to discover any injurious or retarding effect that the sprays might have at a date somewhat earlier than could be expected from trunk circumference measurements. While these data are not presented in detail here, it is perhaps sufficient to state that no significant differences appeared during the 4-year period. The yield data, as presented in figure 2, serve to bear out this point.

It was considered of interest to determine whether or not the spray was influential in reducing leaf area. Accordingly, random 100-leaf samples from each of 5 trees in each treatment were collected and their areas determined photoelectrically. The leaves were collected only from terminals after discarding the 3 oldest and the 3 youngest leaves, so as to retain for measurement only leaves of approximately equal age and like position on the tree. The average areas per leaf in 1936 were 4.80 sq. in. for the check trees, 4.91 sq. in. for the copper-sprayed trees, and 4.83 sq. in. for the sulphur-sprayed trees. In 1937 the averages, in square inches, were, respectively, 5.33 (checks), 5.32 (copper), and 5.34 (sulphur). These figures indicate a significant difference between seasons, but not between treatments in either season. No leaf-area determinations were made after 1937.

In 1936 some determinations of chlorophyll content were made on leaf samples selected as described above. Chlorophyll determinations in mg. per square inch of leaf on July 15 were as follows: unsprayed, 0.578; copper-sprayed, 0.601; lime-sulphur-sprayed, 0.641. On September 8, the same year the checks showed 0.431; copper, 0.410 and sulphur 0.430.

Figure 1 is a graph showing the average trunk circumferences of the trees in each of the 3 lots over the 8-year period. It does not show any pronounced differences, but might be taken to indicate that spraying with either sulphur or copper has resulted in slowing down the average tree growth during the last 4 years in comparison with the unsprayed check trees.

Figure 2 represents the same data plotted to show the average annual increment in trunk circumference rather than the cumulative increase. Figure 2 also shows the average bushel yield per tree. It may be noted that prior to 1937 the trees were growing rapidly, having made in 1936 an average increase in circumference of over 2.25 inches per tree. In 1937 they bore their first crop of fruit. This crop, while small, was associated with and appears to have been the cause of the smaller increment in trunk circumference recorded for that year. In 1938 a heavy freeze in May ruined what promised to be a good crop of fruit. Since the trees did not bear, they were apparently able to make a greater increase in trunk circumference in 1938 than in 1937. In 1939 and 1940 these young trees bore a satisfactory crop for their age, but made correspondingly smaller increases in trunk circumference (Fig. 2). Finally, in 1941, a freeze during the blossoming period again caused a total crop failure, and the rate of tree growth, as measured by circumference, again showed a compensating increase. Varia-

tions in rate and amount of tree growth must necessarily be a reflection of the sum of many factors, but it is believed that these figures show a relationship between the crop of fruit and the amount of growth sufficiently definite

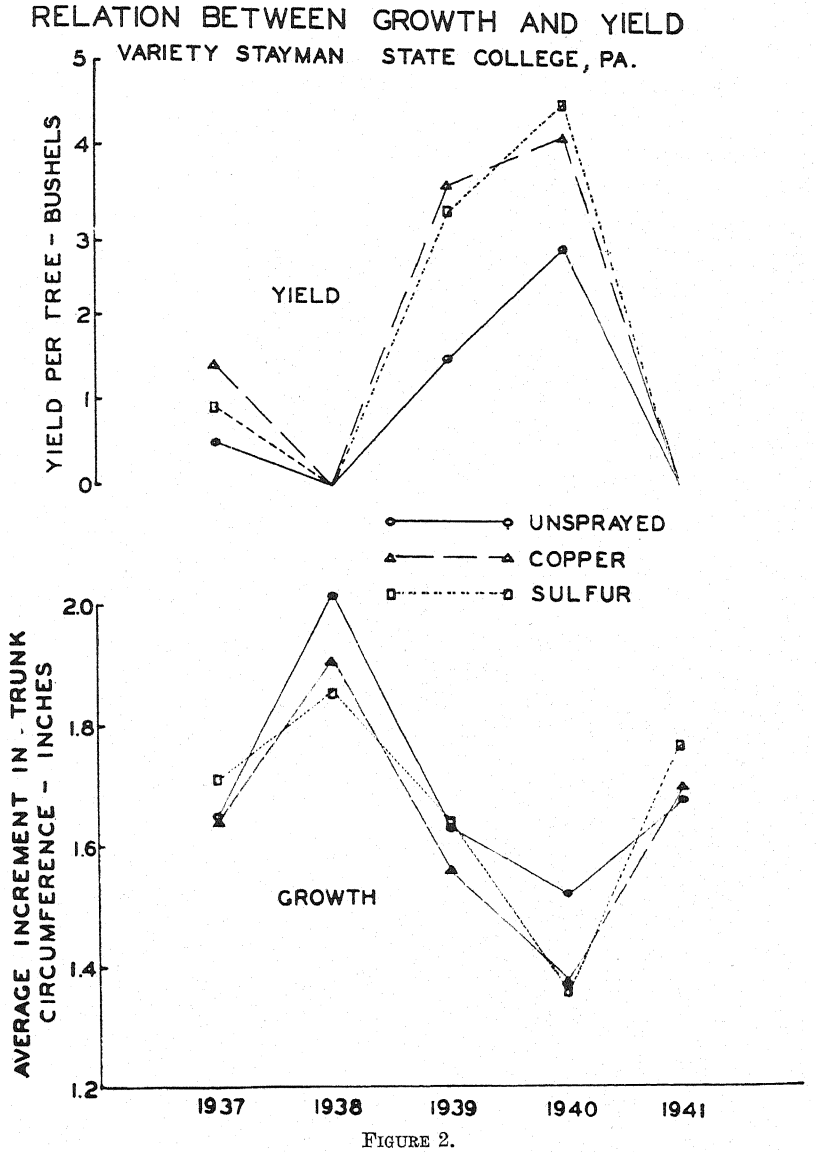



FIGURE 2.

to indicate that the size of the crop must be one of the important factors in determining increase in trunk circumference.

Figure 2 offers a plausible explanation of the tendency indicated in figure 1, where the unsprayed check trees seem to be growing somewhat faster than either lot of sprayed trees. It is believed that this tendency is not

attributable to injury by the spray but is to be correlated instead with the small crop the check trees were able to mature.

In explanation of the small crop produced by the check trees, it should be recorded that in 1937, the first year of bearing, the apples on the checks were 100 per cent scabby, and in 1939 and 1940 only slightly less so. In other words, spraying has served to hold the crop on the trees to maturity, while on the unsprayed trees scab has caused many of the apples to drop off early in the season. This loss of crop is reflected in the increased growth of the unsprayed trees. While there are recognized a great many possible reasons why trees may fail to bear profitable crops or fail to grow in an acceptable manner, we have not been able to demonstrate that spraying is one of them.

THE PENNSYLVANIA STATE COLLEGE,
STATE COLLEGE, PENNSYLVANIA.

# PATHOGENESIS OF DITYLENCHUS DIPSACI IN SEEDLINGS OF ALLIUM CEPA

A. G. NEWHALL[1]

(Accepted for publication April 15, 1942)

## PURPOSE AND METHOD

In a former paper by Newhall and Chitwood (10) the speed with which young onion seedlings become infected with *Ditylenchus dipsaci* from the soil was indicated by the finding of over 50 nematodes in the tissue of 1 seedling 3 weeks after sowing the seed. This has led to a further study of the penetration and tissue preference of this pathogen in young seedlings of Yellow Globe onion.

Home-grown, disease-free seed was sown in 4-in. pots of steamed muck in the greenhouse; and a suspension of *Ditylenchus dipsaci* from chopped, infected onion bulbs containing eggs, larvae and adults, was poured over the top. A constant moisture supply was then maintained for 48 hours by means of a fine mist spray. By the fourth day seeds had begun to germinate in spite of the excessive moisture. Several sprouting seeds were removed, killed in hot 5 per cent formaldehyde solution, and prepared for sectioning by the paraffin method. At this time the sprouts ranged up to 4 mm. in length. Thirty-six hours later a second collection was similarly treated, when many sprouts were 8 mm. in length. A third, a fourth and a fifth collection were all prepared at 2-day intervals thereafter. The mist spray was operated intermittently during this time to keep the soil moist and favor nematode activity. Many seedlings were 2 or 3 mm. above ground at the time of the fifth collection.

Sections from 10 to 20 μ thick were cut and stained in Delafield's aqueous haematoxylin. Fast green in absolute alcohol, alcoholic erythrosin in clove oil, and Bismark brown also were used. The first 3 were the most satisfactory. Sections of the seed failed to adhere to the slides well with the aqueous haematoxylin, though sprouts gave no trouble and seeds adhered well with the alcoholic stains. No difficulty was experienced in cutting through the tough seedcoat in 54° paraffin.

## OBVIOUS TISSUE PREFERENCE OF THIS NEMATODE

Analysis of the many sections revealed several interesting points. Perhaps the most obvious was the almost universal confinement of the attacks to cortical or parenchyma tissue, once the epidermis had been penetrated. Figure 1, A, is a diagrammatic longitudinal section of a germinating onion seed about 6 days after sowing, which gives a composite view of many of the places where nematodes were found. It will be noted they occurred from a point close to the root cap of the hypocotyl (a), to several points within the

[1] The author acknowledges helpful suggestions from Dr. B. G. Chitwood, U. S. Department of Agriculture, Division of Nematology.

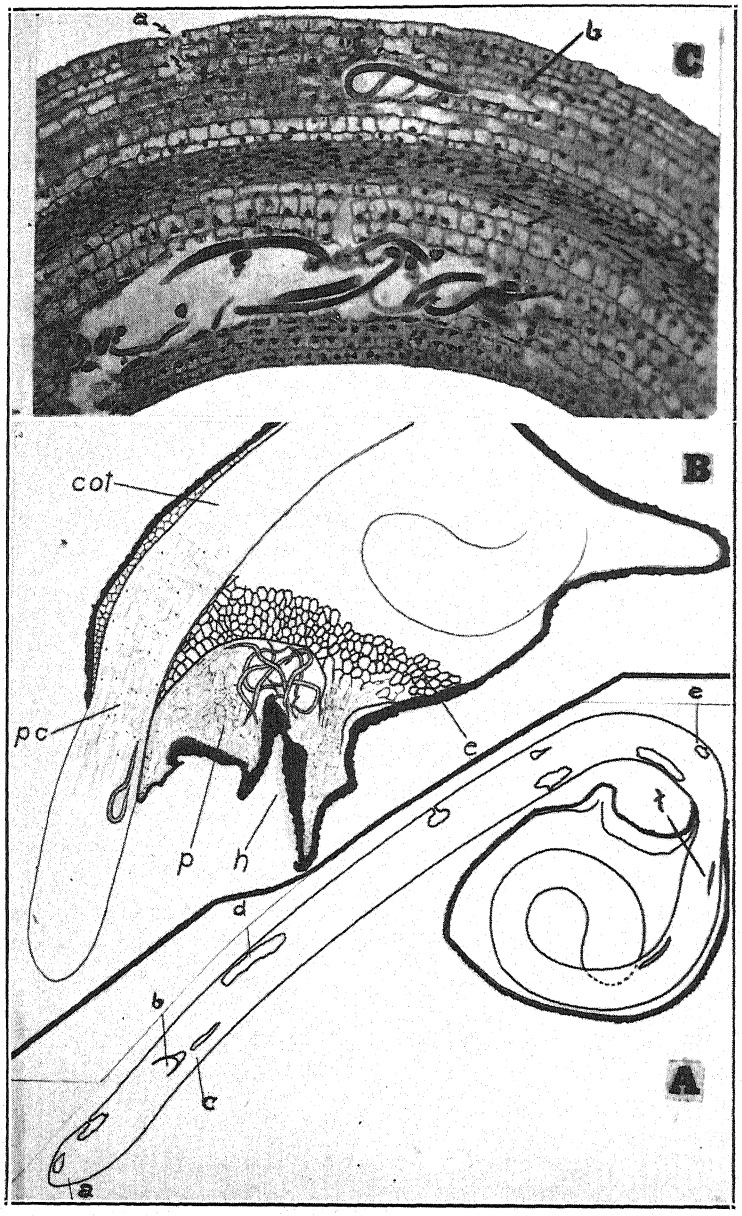



FIG. 1. A. Composite diagrammatic long section through 6-day-old seedling showing places where nemas were found from root tip (a) to within the seed (f). B. Diagrammatic section through seed in early germination to show nemas in soft parenchyma tissue near hilum. cot, cotyledon; pc, procambial strand; p, parenchyma; h, hilum; e, endosperm. C. Photomicrograph of section through cotyledon at point e in A. × 85. a, probable point of entrance of single nema shown above. b, cell recently punctured. Note only slightly damaged endodermal layer of cells protecting central procambium while cortex is heavily invaded. (Stained in aqueous haematoxylin.)

germinating seed (f). In all cases they were found only in cortical or parenchyma tissue. They seemed to be stopped by endodermis surrounding the provascular strand. This is evident also in figure 2, A.

Even in the seed itself their activity was confined to parenchyma tissue.

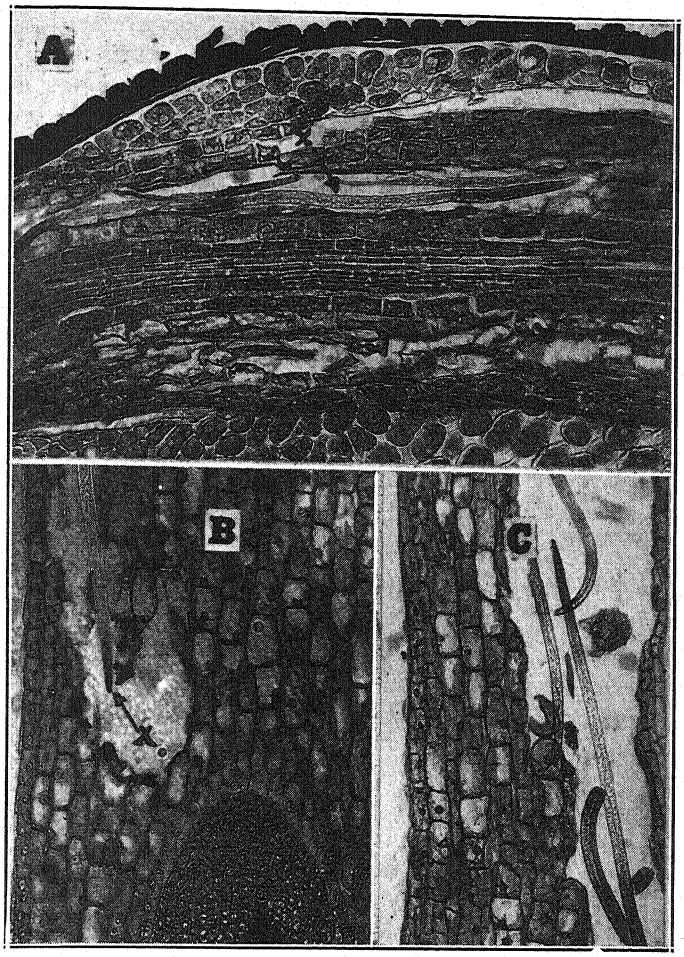



FIG. 2. A. Section through seed, 10 days after germination, at point marked pc in figure 1, B, showing nemas destroying cortex but leaving endoderm surrounding procambium unharmed. B. Nema in cavity of cortex close to primary leaf bud 10 days after germination. C. Nemas in cortex of cotyledon 8 days old. (All × 85; stained in alcoholic erythrosin in clove oil.)

The first collection, made 4 days after sowing, when the sprout was but 2 mm. long, revealed nematodes in two places within the seed. One was in the thin-walled parenchyma just beneath the hilum, through which they perhaps had gained entrance. Their presence here is of interest as indicating how seed transmission, once reported by Ritzema Bos (12), could take place under favorable conditions for migration of larvae up through the parenchyma of

the pedicel prior to seed maturation (Fig. 1, B). The other place in the seed where they were found was within the hypocotyl just before it emerged, as shown in figure 2, A, a section through a germinating seed in the same plane as figure 1, B. At a very early stage in the germination of onion seed there is a well defined single provascular strand. Sometimes, fairly well developed vascular elements can be observed in it. In this section the nematodes can be seen to have broken down the parenchyma tissue on both sides of the procambium, encircling it but in a conspicuous manner avoiding the endodermal layer protecting it. The observation suggests that some secondary wall thickening may take place in the endodermis, even while elongation of the cotyledon occurs. The complete destruction of the parenchyma tissue by the nematode here, as elsewhere in these seedlings, is in striking contrast to their life in the intercellular spaces of the mature onion bulb, and similar mature host tissues such as narcissus bulbs, where they are rarely found within the cells.

One bit of evidence was found that might be assumed to support the theory of secretory action of this nematode on the middle lamella. It is shown in the upper part of figure 1, C, a longitudinal section through the knee of a cotyledon 12 days after germination began. Here an individual may be seen to have forced its head between parenchyma cells for a considerable distance. How much of the resultant separation of the cells is purely mechanical and how much of it has been aided by dissolution of the middle lamella, if there is one at this stage, is not revealed. Mechanical force probably could produce the observed splitting. At the point marked (b) there is evidence of this nematode having broken into the third cell. Further back at the point (a) may be seen the probable point of entrance through a number of definitely ruptured cells.

It is interesting that Godfrey (4), in a study of the relation of *Tylenchus brachyurus* to the root tissue of the pineapple, found that this pathogen also was definitely limited by lignified cells, that, after penetration, all spread was subepidermal, and that even a slight degree of epidermal hardening inhibited primary penetration. Newhall and Chitwood (10) found that mature bulbs of *Allium cepa* were not penetrated in a month by *Ditylenchus dipsaci* in a drop of water unless the skin was first punctured. Laidlaw and Price (6) likewise state that very little infection takes place if healthy onion seedlings (age not given) are transplanted into nematode-infested soil without injuring the epidermis.

### STOMATAL ENTRANCE NOT COMMON

The part played by stomata as portals of entry has received little attention since the announcement of its occurrence in *Vicia faba* by Debray and Maupas (3) and the work on potato by Quanjer (11) in 1927. The finding by the writer of invaded areas in the hypocotyl close to the root cap, where stomates, according to Hoffman (5), are not found, indicates that these portals may not be necessary in the case of onion seedlings (Fig. 1, A, a).

It is true that well formed stomata can be found in the epidermis of the cotyledon soon after germination of the seed begins, but these are closed. As a further test of the role of stomates nematodes were placed in drops of water in Van Tieghem cells on leaves of 10 5-week-old onion plants grown from bulbs. In some cases the epidermis was slightly injured, in others not. In some of the cells cotton or sand was introduced to give the nematodes something to push against. Too much reliance should not be placed on the results of these tests, since they were not repeated, but infection resulted only on the 4 plants the epidermis of which was injured. It is believed that if stomatal penetration of *Allium cepa* by *Ditylenchus dipsaci* occurs at all it is the exception rather than the rule, and that in the case of very young tissue stomates are probably unnecessary as portals of entry.

### RELATION OF AGE OF ONION SEEDLINGS TO SUSCEPTIBILITY

If stomates are not important, if the cuticularizing of the epidermis is, and if this takes place soon after germination of the seed, it follows that onions should become more resistant as the seedlings grow older. Accordingly, in the following experiment 3 replicate plantings of 100 seeds of Yellow Globe onion were sown every 5 days in loam soil until 5 plantings had been made. Ten days after the last sowing, when the oldest were 30 days of age, all were inoculated by pouring over the rows a measured volume of a water suspension of *Ditylenchus dipsaci* obtained by crushing heavily infested onion bulbs. This inoculum contained all stages in the life of the nematode, including many eggs. Heavy infection (over 80 per cent) took place on the 10-day-old seedlings, 18 per cent on those 15 days old, while less than 7 per cent and 4 per cent occurred on the 25- and 30-day-old plants, respectively. This experiment was repeated, using muck soil, with substantially similar results. These are presented in table 1 where the mean numbers of healthy seedlings are considered as an inverse measure of the infective capacity of the nematode. Infected seedlings were counted but not frequently enough to keep up with their death and disappearance. From a glance at the data obtained on the 38th day it appears that many of the older plants were late in showing distinct symptoms. Even so it remains evident that a marked reduction in susceptibility seems to occur in seedlings between the 10th and 20th days. This trend is in the opposite direction from that to be expected if stomates functioned as the chief portals of entry, because they would have been getting larger during this time.

These results check closely with field observations. Much greater losses are believed to occur if seed is sown on infested areas than if sets are planted. Sets are by no means immune however, perhaps no more so than 30-day-old seedlings. In the field there is opportunity for nematodes to be spattered up into the crotches between the new leaves, where they would come in contact with tender young epidermal tissue. In seasons lacking rainfall, losses are much reduced.

The results of these 2 experiments tend to support Anderson's (1) sug-

TABLE 1.—*Effect of age of onion seedlings on their capacity to resist penetration of Ditylenchus dipsaci in muck soil*[a]

| | Number of healthy seedlings surviving 7 weeks period | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Check | Inoc. | Check | Inoc. | Check | Inoc. | Check | Inoc. | Check | Inoc. | Mean No. | |
| Age of seedlings, in days, at time of inoculation | 10 | 10 | 15 | 15 | 20 | 20 | 25 | 25 | 30 | 30 | Check | Inoc. |
| Healthy seedlings on day of inoculation | 81 | 88 | 86 | 82 | 82 | 83 | 86 | 94 | 82 | 86 | 83 | 86 |
| Healthy seedlings 24 days after inoculation | 78 | 31 | 83 | 52 | 82 | 68 | 80 | 73 | 81 | 70 | 81 | 59 |
| Healthy seedlings 38 days after inoculation | 77 | 3 | 82 | 11 | 78 | 43 | 77 | 54 | 79 | 53 | 79 | 33 |

[a] Data obtained by B. T. Goulko under the writer's direction and used with his permission.

gestion that very early in the life of *Allium cepa* the epidermis undergoes a hardening process that progressively fortifies it against penetration by the smut fungus, *Urocystis cepulae*. Dr. Arzberger, according to Steiner (13) found evidence which led him to believe that the Iron and Brabham varieties of cowpea owe their resistance to the root-knot nematode to the fact that their roots are better guarded by protective tissue than susceptible varieties. The layer of cork cells is better developed and has more suberized walls, and, in general, the mechanical tissue is more uniformly distributed through the cortex in the resistant varieties. The more recent work of Barrons (2), to be sure, throws doubt on the importance of mechanical resistance to *Heterodera marioni* in seedlings of certain beans and cowpeas, since he was able to find as many nematodes in the root tips of the resistant as those of susceptible strains. No such search was undertaken in the present study, though, admittedly, it might have been revealing.

## DISCUSSION

Swelling of invaded tissue has been considered a prominent characteristic of onion bloat on seedlings, as pictured by Newhall and Chitwood (10), but in these studies on paraffin sections of very young seedlings, up to 12 days of age, this symptom was not prominent. Neither was there much evidence of swelling of individual cells or of discoloration in advance of the pathogen, as noted by Quanjer (11) in the shoots of potatoes. There was very little time for secondary organisms to affect the picture, and no evidence of their action could be found. One is tempted to ask whether this fact could be responsible for the paucity of evidence indicating dissolution of the middle lamella. Evidence of such dissolution admittedly may have been obscured by the processing that preceded the microscopical examination of the sections.

One prominent histologic symptom seen in figure 2 was the large number of host cells in the vicinity of the heads of the nematodes that have lost all or a portion of their contents. This may be significant in view of the recent observations on the feeding habits of living hollow-stylet nematodes made by Linford, (8, 9) and Linford and Olivera (7). The conclusion they reached was, contrary to the views formerly expressed by others, that *Ditylenchus dipsaci,* like a number of predacious, hollow-stylet nematodes, feeds on its host by puncturing the cells and sucking out their contents. The predacious forms, such as *Ditylenchus intermedius* and some of the Aphelenchoides, were observed to inject powerful digestive fluids into their prey, which paralyzed them quickly and reduced their body contents to a fluid. This was easily withdrawn through the narrow stylet by the pulsating action of the esophageal bulb. *D. dipsaci* also was observed by Linford to jab its stylet through the wall of a fungus and apparently to feed on the contents by this same action, suggesting that soil fungi might constitute the food of this plant pathogen in years when normal plant hosts were lacking. The destruction of the parenchyma tissue observed in onion seedlings and pictured in the

accompanying illustrations is manifestly what would be expected from feeding activity of this kind.

In conclusion, these studies indicate that *Ditylenchus dipsaci* does not feed on the cells of young onion seedlings by dissolving the middle lamella, although it may possibly have such capacity. This nematode apparently feeds more often by the method described by Linford involving repeated piercing of the cell walls of the parenchyma and feeding on the cell contents until the cells are so weakened that they collapse under the pressure of the body activities of the pathogen. Migration is typically not between but more often right through the broken cells (Fig. 2). They have been found destroying the closely packed meristematic cells of the young leaf primordium and of the root tip. These lack intercellular spaces but doubtless have cell walls in a more or less fluid state, relatively easy to puncture.

## SUMMARY

A study was made of paraffin sections of onion seedlings up to 12 days of age removed at 2-day intervals from soil, heavily infested with *Ditylenchus dipsaci*. The nematode was found to penetrate the seed soon after germination commenced and to live for a short time in the parenchyma tissue beneath the hilum or in that of the cotyledon.

Penetration of the cotyledon after its protrusion apparently could take place directly through the young epidermis at any point. Once within the sprout, migration occurred freely through the tender cortical tissue both longitudinally and radially but was definitely bounded by dermal layers even at early stages in germination. No evidence of penetration of the provascular strand was observed.

Evidence supporting the theory that this nematode migrates only between the cells by dissolving the middle lamella was meagre and not considered convincing. But evidence of destructive mechanical intracellular penetration of parenchyma tissue was abundant.

Further evidence is presented indicating that as seedlings reach the age of 3 weeks they become more resistant to attack by nematodes in the soil. It is suggested that this may be due to probable increased hardening of the epidermis.

## LITERATURE CITED

1. ANDERSON, P. J. Development and pathogenesis of the onion smut fungus. Mass. Tech. Bul. 4: 99–133. 1921.
2. BARRONS, KEITH C. Studies on the nature of root-knot resistance. Jour. Agr. Res. [U. S.] 58: 263–271. 1939.
3. DEBRAY, F., and E. MAUPAS. Le Tylenchus devastatrix Kühn, et la maladie vermiculaire des féves en Algerie. L'Algerie agricola, 1–55. 1896.
4. GODFREY, G. H. A destructive root disease of pineapples and other plants due to *Tylenchus brachyurus* n. sp. Phytopath. 19: 623–629. 1929.
5. HOFFMAN, C. A. The developmental morphology of *Allium cepa*. Bot. Gaz. 95: 279–299. 1933.
6. LAIDLAW, W., and C. A. PRICE. The onion eelworm. Victoria Dept. Agr. Jour. 8: 163–171. 1910.
7. LINFORD, M. B., and J. M. OLIVEIRA. The feeding of hollow spear nematodes on other nematodes. Science 85: 295–297. 1937.

8. LINFORD, M. B. The feeding of some hollow-stylet nematodes. Helminth. Soc. Wash. Proc. 4: 41–46. 1937.
9. ————. Notes on the feeding of *Ditylenchus dipsaci*. (Nematoda : Tylenchidae.) Helminth. Soc. Wash. Proc. 4: 46–47. 1937.
10. NEWHALL, A. G., and B. G. CHITWOOD. Onion eelworm rot or bloat caused by the stem or bulb nematode, *Ditylenchus dipsaci*. Phytopath. 30: 390–400. 1940.
11. QUANJER, H. M. Een aaltjesziekte van de aardappelplant, de aantastingswijze en de herkomst van haar oorzaak, *Tylenchus dipsaci* Kühn. Tijdschr. Plantenziekt 33: 137–172. 1927.
12. RITZEMA BOS, J. Untersuchungen über Tylenchus devastatrix Kühn. Biol. Centr. 7: 646–659. 1888. Biol. Centr. 8: 129–138, 164–178. 1888.
13. STEINER, G. The problem of host selection and host specialization of certain plant infecting nemas and its application in the study of nemic pests. Phytopath. 15: 499–534. 1925.

# PHYSIOLOGIC SPECIALIZATION IN CERCOSPORA ORYZAE

T. C. Ryker

(Accepted for publication April 10, 1942)

While selecting strains of Blue Rose rice, resistant to *Cercospora oryzae* Miy., it was observed that inbred lines, ordinarily resistant to the fungus, were susceptible when inoculated with certain cultures. This indicated the existence of races of the fungus that differed in pathogenicity. It is the purpose of this paper to report data showing that physiologic races occur in *Cercospora oryzae*. A brief abstract of these results have been published.[1]

## MATERIALS AND METHODS

Tissue isolates of the fungus were used in most cases for the inoculations. These were obtained by plating individual lesions from naturally infected leaves, surface-sterilized with a 1:1000 corrosive sublimate solution in 50 per cent ethyl alcohol. A few monoconidial cultures also were used. To obtain the inoculum, streaks were first made on bean-agar slants from cultures 3 to 6 days old. After incubating the slants for 4 to 6 days, sterile water was added and a spore suspension was secured by scraping the surface of the medium with a needle. This suspension was poured on bean agar plates and the excess removed. After 3 to 6 days water was added and the surface of the agar was brushed with a stiff camel-hair brush to loosen the spores. Five plates made approximately 1 pint of the spore suspension required for inoculation.

Two apparently equally effective methods of inoculation were employed. (1) The plants were sprayed with the spore suspension, placed in a moist chamber for 24 to 48 hours, and then put in the open. (2) The plants were left in the open and were sprayed between sundown and dark. It was found that satisfactory infection could be obtained only in the late spring, summer, and early autumn. Satisfactory infection was not obtained if the plants were kept in the greenhouse following inoculation.

The plants were grown in 2-gallon glazed earthenware crocks and were inoculated when approximately 12 inches high. Repeated inoculations have shown no apparent difference in reaction of seedlings and mature plants to the fungus.

## EXPERIMENTAL RESULTS

In a preliminary inoculation experiment, 20 isolates of the fungus were tested on the rice varieties Fortuna, Blue Rose, Caloro, and Blue Rose 41. The host reactions were relatively clear-cut. On a plant susceptible to a culture, the spots appeared in 10 to 12 days and slowly enlarged until they were 7 to 10 mm. long. On a resistant plant the lesions usually did not appear before the 18th day, and remained quite small. An intermediate

[1] Ryker, T. C. Physiological specialization in *Cercospora oryzae*. (Abstract) Phytopath. 30: 21. 1940.

reaction was observed with some varieties when inoculated with certain cultures; on these varieties, which are being considered as moderately resistant, the incubation period was 2–3 days longer than in the case of the susceptible plants. The spots never exceeded ½–⅔ the length of the lesions on susceptible plants. The results of this experiment (Table 1) show that at least

TABLE 1.—*Reaction of 4 varieties of rice to 20 cultures of Cercospora oryzae*

| Race No. | No. of cultures tested | Host from which cultures were obtained | Varieties inoculated[a] | | | |
|---|---|---|---|---|---|---|
| | | | Blue Rose | Blue Rose 41 | Fortuna | Caloro |
| 1 | 5 | Blue Rose | S | R | R | R |
| | 1 | Unknown | S | R | R | R |
| | 1 | Delitus | S | R | R | R |
| | 1 | Early Prolific | S | R | R | R |
| | 2 | Blue Rose 41 | S | R | R | R |
| 2 | 3 | Blue Rose 41 | MR | S | R | R |
| | 6 | Unknown | MR | S | R | R |
| 3 | 1 | Caloro | S | R | R | S |

[a] S = Susceptible; MR = Moderately Resistant; R = Resistant.

3 distinct pathogenic races of the fungus exist: race 1, to which Blue Rose was susceptible and Blue Rose 41, Fortuna, and Caloro were resistant; race 2 to which Blue Rose was moderately resistant, Blue Rose 41 was susceptible, and Fortuna and Caloro were resistant; and race 3, which differed from the first in that Caloro was susceptible.

Nineteen rice varieties were then inoculated with the 3 races of the fungus and with a number of other previously isolated cultures, in order to determine the number of physiologic races present in the various cultures, to select as few varieties as possible for use in identifying the different races, and to determine the reaction of the commercial rice varieties to be employed in breeding work. The varieties Blue Rose, Blue Rose 41, Fortuna, Caloro, Colusa, Zenith, Delitus, and Southern Red Rice proved useful in separating the several races. The varieties Rexoro, Nira, Iola, Kameji, and Shoemed were resistant to all races of the fungus. The varieties Honduras, Edith, and Carolina Gold were susceptible to most races of the fungus. The variety Vintula was apparently not homozygous for resistance as a few of the individuals were susceptible.

Another series of inoculations was made on the same 8 varieties selected as race differentials in the earlier experiments. In all, 36 isolates of the fungus were tested for pathogenicity on these varieties. The results, summarized in table 2, show that 5 clear-cut races could be differentiated on the basis of the reaction of the rice varieties Blue Rose, Blue Rose 41, Fortuna, and Caloro. When Colusa, Zenith, and Delitus were added as test varieties, race 1 could be fuurther subdivided into 5 subraces, race 2 into 3, and race 3 into 6, making a total of 16 pathogenic races among the 36 isolates tested. It is probable that more races could be differentiated by the addition of more

TABLE 2.—*Reaction of 8 varieties of rice to 36 cultures of Cercospora oryzae*

| Race No. | No. of cultures tested | Host from which cultures were obtained | Varieties inoculated[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Blue Rose | Blue Rose 41 | Fortuna | Caloro | Colusa | Zenith | Delitus | Southern Red Rice |
| 1 | 10 | 4 Blue Rose, 1 Blue Rose 41, 1 Delitus, 1 Fortuna, 1 Vintula, 1 Caloro, 1 Colusa | S | R | R | R | S | S | S | R[b] |
| 1–a | 3 | Blue Rose, Vintula, Edith | S | R | R | R | R | S | S | ..... |
| 1–b | 5 | 2 Blue Rose 41, 1 Vintula, 1 Delitus, 1 Acadia | S | R | R | R | R | S | R | ..... |
| 1–c | 2 | Zenith, Colusa | S | R | R | R | MR | R | R | ..... |
| 1–d | 1 | Blue Rose | S | R | R | R | S | R | S | ..... |
| 2 | 1 | Unknown | MR | S | R | R | R | MR | MR | R |
| 2–a | 1 | Blue Rose 41 | S | S | R | R | MR | MR | MR | ..... |
| 2–b | 1 | Blue Rose 41 | MR | S | R | R | MR | MR | R | ..... |
| 3 | 2 | Caloro, Edith | S | R | R | S | MR | S | MR | R |
| 3–a | 1 | Caloro | S | R | R | S | R | R | MR | ..... |
| 3–b | 2 | Edith, Calady | S | R | R | S | S | R | R | ..... |
| 3–c | 1 | Blue Rose 41 | S | R | R | S | S | S | R | ..... |
| 3–d | 1 | Blue Rose 41 | S | R | R | S | R | R | R | ..... |
| 3–e | 1 | Wataribune | S | R | R | S | S | R | S | ..... |
| 4 | 2 | Fortuna | R | R | S | R | R | MR | MR | ..... |
| 5 | 2 | Southern Red Rice | R | R | R | R | R | MR | MR | S |

[a] R = resistant; S = susceptible; MR = moderately resistant.
[b] Southern Red Rice was not tested with all cultures of this group.

varieties to the differential hosts. However, for practical importance in plant breeding, the reaction of Fortuna, Blue Rose, Blue Rose 41, and Caloro is the most important. For this reason, the designation of races was made on the basis of these four varieties. It may be seen in table 2 that race 2-a

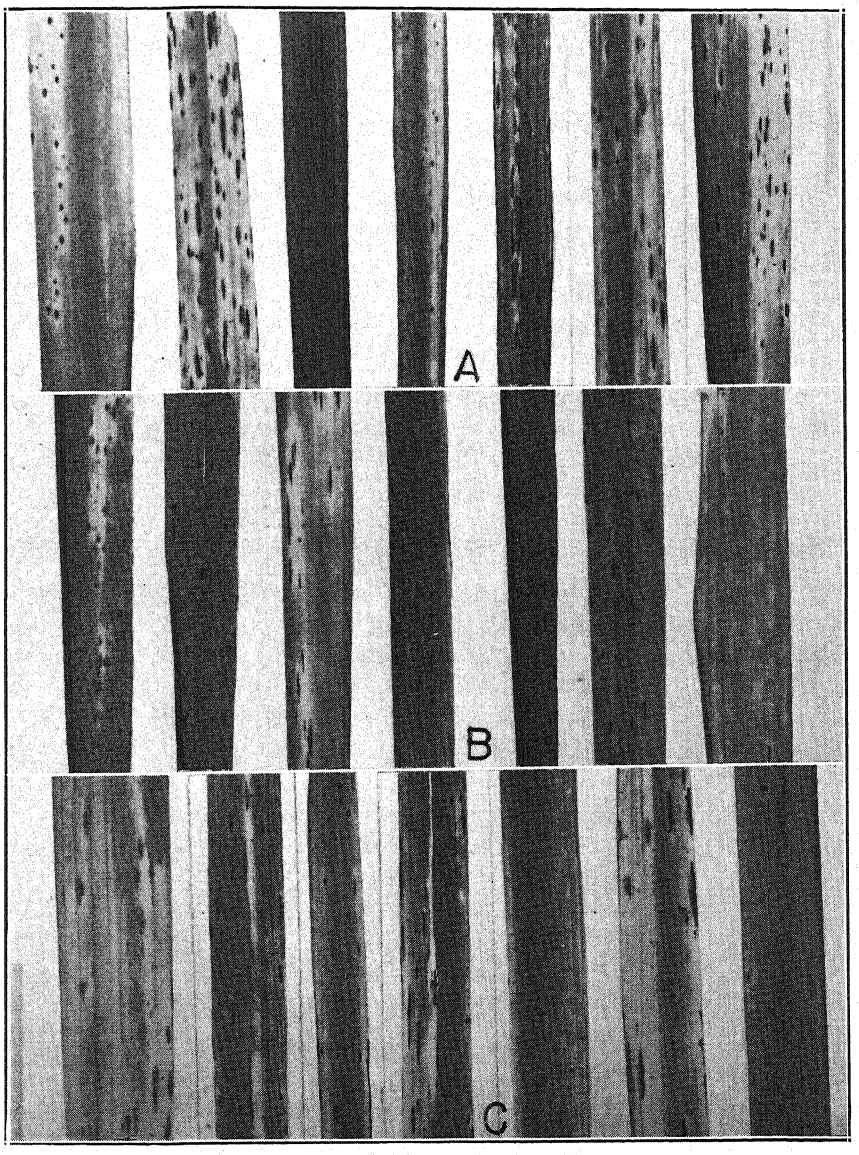



FIG. 1. Reaction of rice varieties to three races of *Cercospora oryzae*. A. Race 1, B. Race 2, C. Race 3. Varieties reading from left to right: Fortuna, Blue Rose, Blue Rose 41, Caloro, Colusa, Zenith, and Delitus.

differed somewhat from race 2 on Blue Rose and Caloro but this difference was not considered sufficient to make it a separate race. In figure 1 are shown the reactions of 7 test varieties to races 1, 2, and 3.

Attention should be called to the reaction of Southern Red Rice, a very objectionable weed in commercial rice fields. In most fields it is resistant to *Cercospora oryzae,* but where it has gained a foothold because of poor cultural practices it may become severely diseased because of the increase of the pathogenic race to which it is susceptible.

## SUMMARY

The occurrence of 5 definite races of *Cercospora oryzae* and a number of others considered as sub-races, differing in pathogenicity on 8 differential host varieties, is reported.

Certain rice varieties have been found resistant to all known races of the fungus.

Blue Rose, the variety most widely planted in Louisiana, is susceptible to most of the races known to occur in the State.

A selection of Blue Rose (Blue Rose 41) is resistant to race 1, the most common race in the rice-growing district of Louisiana.

DEPARTMENT OF PLANT PATHOLOGY,
LOUISIANA AGRICULTURAL EXPERIMENT STATION,
BATON ROUGE, LOUISIANA.

## PHYTOPATHOLOGICAL NOTES

*A Simple Technique for Isolating Spores of Various Fungi from Exposed Slides in Aerobiological Work.*[1]—One of the problems confronting aerobiologists is that of identifying fungus spores found on exposed slides. It is relatively simple to identify spores of some fungi, such as *Alternaria* and *Helminthosporium,* because the morphological characters are distinctive for the genus, but it often is impossible to make specific determinations even of such fungi. On the other hand, it is impossible to identify spores of many genera, such as *Aspergillus* and *Penicillium,* on the basis of their morphological characters only. A method of determining the identity of spores caught on slides would be of great value in studies on wind dissemination of plant-disease fungi as well as those that are allergenic to human beings. The writer has applied the following simple technique, which has aided to some extent in solving this problem.

Under low power of the microscope, location of a spore or group of spores on the slide was marked with a crow-quill pen and India ink. Then the slide was inverted over a Van Tieghem cell and single spores were isolated by means of a micromanipulator, according to the method described by Hanna.[2] If the spores germinated and produced sporulating cultures they could be identified because the manner of spore formation, an important character in the identification of fungi, was known.

The writer first applied this technique in attempting to identify small, ovoid, brown spores, present in thousands on slides (3 sq. in.) that had been coated with vaseline and exposed to catch rust spores. The ovoid, brown spores, 5–9 μ in diameter, germinated after they were isolated by the above method. Since, on germinating, they produced promycelia with hyphal branches, it was concluded that they were spores of one of the loose smuts, either *Ustilago nuda* or *U. tritici.* Since then, the spores of many other fungi, including *Penicillium, Aspergillus, Trichoderma, Cephalosporium, Fusarium* (micro-conidia), *Cladosporium, Rhizopus,* and *Pleospora,* have been isolated and similarly identified. The spores of none of the above fungi are morphologically distinctive enough to enable one to identify them with any degree of certainty, yet it is relatively simple to identify cultures of these fungi.

Obviously, the method described has limits within which it can be used. The spores to be identified must be viable and capable of growing on artificial media, and the cultures must sporulate in order to make possible the identification. The writer has had little difficulty in getting isolated spores to germinate, although some of them failed to do so. The length of time between exposure of slide and attempts to germinate spores is an important

[1] Paper No. 2029 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Hanna, W. F. A simple apparatus for isolating single spores. Phytopath. 18: 1017–1021. 1928.

factor; yet, smut spores on slides 3 months old were viable, and spores of *Fusarium* were viable as long as a month after exposure of the slide. Only a few of the cultures obtained from the slides failed to sporulate.

The advantages of using the method described rather than that of washing the spores from a slide with sterile water and then making dilution plates, is that the identity of a particular spore or group of spores can be determined by this method. Furthermore, the difficulty of having slow-growing fungi obscured by fast-growing forms is avoided.—W. J. MARTIN, University Farm, St. Paul, Minnesota.

*Distribution of Beauveria bassiana on Elm Insects in the United States.*—In 1941 and 1942 a survey was made for the presence of *Beauveria bassiana* (Bals.) Vuill. on species of insects found on elms. In 1941, Charles[1] reported its occurrence on *Scolytus multistriatus* Marsham in New Jersey. The occurrence of *B. bassiana* in Pennsylvania and New Hampshire is recorded in this report. In addition, the fungus has been isolated from the adults of several additional elm insects, namely, *Hylurgopinus rufipes* (Eich.), *Magdalis barbita* (Say), *M. armicollis* (Say), *Saperda tridentata* Oliv., and *Tremex columba* (L.). All but the latter are elm borers or bark beetles. *Hylurgopinus rufipes* (Eich.) is considered the next most important to *S. multistriatus* in transmitting the Dutch elm disease pathogen. *Ceratostomella ulmi* has been isolated from the other insects listed, except *Tremex columba* (L.), but they are not at this time considered important in the dissemination of the pathogen.

The distribution of *Beauveria bassiana* and the insect hosts of the fungus as determined in this study are recorded in table 1. Negative results are included to indicate species collected and range and extent of collections. In all cases, identification was made on the basis of cultural characters after careful comparison with an authentic strain of *B. bassiana*.[2]

The above table reveals that approximately 3 per cent of all insects cultured carried *Beauveria bassiana*. As these insects were cultured by the method found useful in isolating *Ceratostomella ulmi*, it is likely that a more suitable method for obtaining *B. bassiana* would have yielded a higher percentage.

The Charles check list[3] reported *Beauveria bassiana* from Maine to Florida and from the Atlantic to the Pacific Coast States. Twenty-nine species or insect groups were given as hosts for *B. bassiana* from 17 States besides the District of Columbia, Canada, and the Dominican Republic. The present report also indicates that *B. bassiana* is widely distributed and found on numerous hosts. That it has not been reported previously in some of the States and on many other insects is undoubtedly due to the lack of

[1] Charles, Vera K. A preliminary check list of the entomogenous fungi of North America. Insect Pest Surv. Bull. 21: suppl. to No. 9, 707–785. 1941.

[2] For comparative study an authentic strain of *B. bassiana* was supplied by E. G. Rex and E. E. McCoy of the New Jersey State Department of Agriculture.

[3] See footnote 1.

TABLE 1.—*Geographical distribution of Beauveria bassiana on elm insects cultured in 1941–42 at the Forest Pathology Field Laboratory, Morristown, N. J.*

| Insects from | Host | Number of insects | | Percentage positive |
|---|---|---|---|---|
| | | Cultured | Positive | |
| District of Columbia | *Scolytus multistriatus* Marsham | 14 | 0 | ..... |
| Indiana[a] | do | 94 | 0 | ..... |
| Kentucky | do | 19 | 0 | ..... |
| Maryland | do | 40 | 0 | ..... |
| do | *Platysoma coarctatum* Lec. | 4 | 0 | ..... |
| Massachusetts | *S. multistriatus* Marsham | 8 | 0 | ..... |
| do | *Hylurgopinus rufipes* (Eich.) | 17 | 3 | 17.6 |
| New Hampshire | do | 92 | 12 | 13.0 |
| New Jersey | do | 1377 | 39 | 2.8 |
| do | *S. multistriatus* Marsham | 3647 | 95 | 2.6 |
| do | *Magdalis barbita* (Say) | 87 | 1 | 1.1 |
| do | *M. armicollis* (Say) | 24 | 1 | 4.2 |
| do | *Saperda tridentata* Oliv. | 2 | 0 | ..... |
| do | *Tremex columba* (L.) | 1 | 1 | 100.0 |
| New York | *H. rufipes* (Eich.) | 70 | 0 | ..... |
| do | *Saperda tridentata* Oliv. | 20 | 10 | 50.0 |
| Ohio | *S. multistriatus* Marsham | 43 | 0 | ..... |
| Pennsylvania | do | 52 | 1 | 1.9 |
| do | *H. rufipes* (Eich.) | 15 | 3 | ..... |
| Virginia | *S. multistriatus* Marsham | 12 | 2 | 16.6 |
| Vermont | *H. rufipes* (Eich.) | 18 | 0 | ..... |
| | Total | 5656 | 168 | 3.0 |

[a] One case of *B. bassiana* was observed but attempts to isolate the organism in pure culture failed.

attempts to find it, or that insufficient numbers of insects have been cultured to reveal its presence.—PAUL V. MOOK and D. O. WOLFENBARGER, Division of Forest Pathology, Bureau of Plant Industry, and Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Cooperating.

*Effect of Cyanide on Synthesis of Ring-Spot and Mosaic Viruses in Tobacco.*[1]—Ring-spot virus (*Annulus tabaci* H.), and tobacco-mosaic virus (*Marmor tabaci* H.), differ markedly in their stabilities and chemical properties.[2] Because of these differences it was of interest to compare the effects of cyanide on the two viruses *in vivo*. Preliminary tests indicated that treatments that inhibited synthesis of tobacco-mosaic protein[3] also blocked the formation of ring-spot protein. Further studies have aided in elucidating the fundamental processes involved in the multiplication of these viruses in living cells and are suggestive with respect to the nature of the "acquired immunity" that develops following infection with ring spot.

Experiments were conducted with an inbred strain of *Nicotiana tabacum* L. var. Turkish, which shows considerable necrosis following ring-spot infec-

[1] Scientific Paper Number A34, Contribution No. 1855, of the Maryland Agricultural Experiment Station (Department of Botany).

[2] Stanley, W. M. The isolation and properties of tobacco ring spot virus. Jour. Biol. Chem. 129: 405–428. 1939.

[3] Woods, M. W. Reversible inhibition of tobacco mosaic virus in living cells with 0.0002 molar sodium cyanide. Science 91: 295–296. 1940.

tion, a Maryland commercial strain of *N. tabacum,* which likewise responds necrotically to ring spot, and a vegetatively propagated hybrid clone derived from Turkish tobacco and *N. glutinosa.* This hybrid carries Holmes'[4] dominant gene for necrotic response to tobacco-mosaic virus, but is only slightly necrotized by ring spot. Strains of green- and yellow-mosaic viruses, used in previous work,[5] were employed. The ring-spot virus was obtained two years ago from W. C. Price and has been maintained in Turkish tobacco. To avoid variability due to genetic or physiological factors, single test leaves were cut into sectors and each sector exposed to a particular treatment. Immediately after inoculation, leaf sectors were floated, dorsal surface up, on appropriate solutions in shallow (1 cm.) dishes for at least 12 to 14 hours before cyanide treatments were begun. Nutrient solutions, with or without cyanide, were supplied from 22-l. bottles by constant drip from capillary tubes. All tests were run in the greenhouse under conditions favorable for growth of tobacco. Four set-ups, each accommodating 8 leaf sectors, were employed. At least 4 leaves were exposed to each treatment. It was thus possible to follow simultaneously the development of lesions induced by 2 viruses in a single leaf under 4 different sets of experimental conditions. Results were readily reproducible. 0.00015 to 0.0003 molar KCN reversibly blocks from 50 to 80 per cent of respiration in the tobacco leaf,[6] and reversibly inhibits the synthesis of tobacco mosaic virus protein.[5] The same concentrations were found to inhibit the development of primary lesions of ring spot. Since the formation of necrotic areas in ring spot is markedly influenced by the environment[7] the development of visible lesions

TABLE 1.—*Effect of alternating treatments with 0.0003 molar KCN on time of appearance of primary lesions of ring spot and two strains of tobacco mosaic, with and without external nitrogen supply. Cyanide treatments discontinued 65½ hours after inoculation in mosaic experiments and 92 hours after inoculation in ring-spot experiment*

| Virus[a] | Average number of lesions visible per square inch | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 100 p.p.m. of N; no KCN | | 100 p.p.m. of N; 0.0003 M. KCN | | Minus N; no KCN | | Minus N; 0.0003 M. KCN | |
| | Hours after inoculation | | Hours after inoculation | | Hours after inoculation | | Hours after inoculation | |
| | 65 | 141 | 65 | 141 | 65 | 141 | 65 | 141 |
| Green mosaic | 20.6 | 25.8 | 5.1 | 25.2 | 14.2 | 20.7 | 7.0 | 29.8 |
| Yellow mosaic | 10.3 | 15.5 | 1.6 | 20.1 | 10.4 | 12.7 | 2.3 | 17.0 |
| Ring spot[b] | 20.9 | 26.2 | 7.6 | 21.0 | 21.6 | 27.1 | 11.5 | 26.1 |

[a] Mosaic tests in *N. tabacum* × *N. glutinosa* hybrid and ring-spot tests in *N. tabacum.*
[b] Ring-spot lesion counts made 98 and 164 hours after inoculation instead of 65 and 141 hours, respectively.

[4] Holmes, F. O. Genes affecting response of *Nicotiana tabacum* hybrids to tobacco mosaic virus. Science 85: 104–105. 1937.
[5] Woods, M. W., and H. G. DuBuy. Synthesis of tobacco mosaic virus protein in relation to leaf chromoprotein and cell metabolism. Phytopath. 31: 978–990. 1941.
[6] Woods, M. W., and H. G. DuBuy. The effect of tobacco mosaic virus on cellular respiration. Phytopath. 32: 288–302. 1942.
[7] Woods, M. W. Cellular changes in ring-spot. Contrib. Boyce Thompson Inst. 6: 51–67. 1934.

alone cannot be taken as the only criterion of virus formation. Biological assays for virus (local lesion counts on Turkish tobacco half leaves), however, demonstrated that cyanide not only suppresses necrosis in ring spot (Fig. 1), but actually inhibits synthesis of virus as well. For example, in one experiment with *Nicotiana tabacum,* in which the ring-spot-inoculated sectors were treated with 0.0003 molar KCN for 24.0 per cent of the total time, a 55 per cent reduction in virus titre occurred. In another similar test these values were 22 per cent and 41 per cent, respectively. The retarding effect of cyanide on development of primary lesions induced by ring spot, and the two strains of tobacco mosaic, are illustrated in table 1. This retarding effect is reversible as evidenced by enlargement of the lesions (Fig. 1) and the rise in virus titre following cessation of treatments with KCN. The same concentrations of cyanide also block the "A" and "B" systems[6] of oxygen respiration.

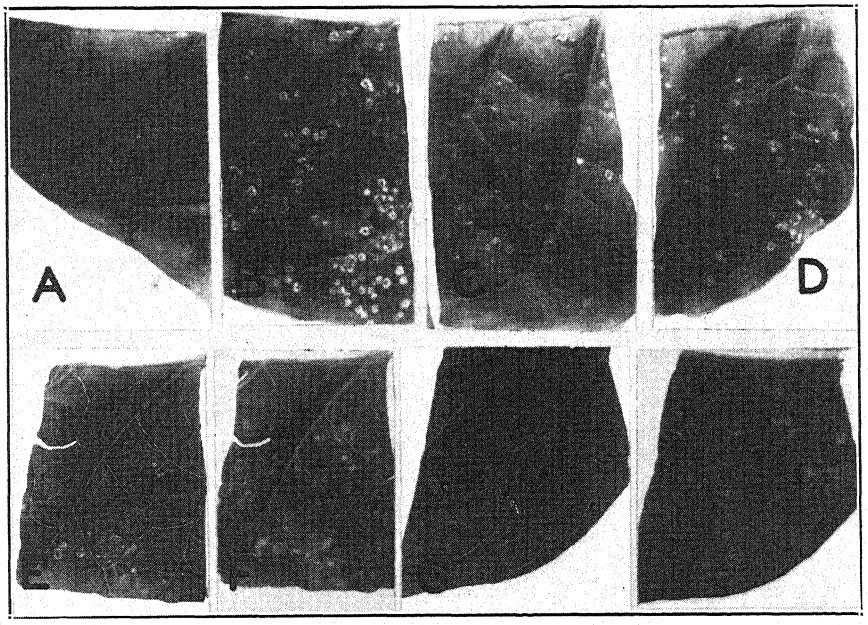



FIG. 1. Influence of KCN on development of primary lesions of ring spot in *Nicotiana tabacum.* A–D. Four sectors in one leaf of Turkish tobacco 95 hours after inoculation. A. 0.0003 molar KCN applied at night only. B. No cyanide. C. 0.0003 molar KCN applied by day only. D. No cyanide. E. Maryland commercial tobacco, untreated, 98 hours after inoculation. F. Same sector as in E, but 167 hours after inoculation. G. Sector of same leaf as in E, 98 hours after inoculation, but including 35 hours of treatment with 0.0003 molar KCN. H. Same sector as in G, 167 hours after inoculation and 65 hours after cessation of cyanide treatments.

When cyanide was administered *at night only* ring-spot lesions were much less necrotic and of lower virus titre than in the controls (not cyanided), or in leaf sectors treated with cyanide *during the day only.* In some instances complete masking of symptoms resulted (Fig. 1). *Such "protected" infected tissues continued to resist necrosis following cessation*

*of cyanide treatments.* In sectors treated with *cyanide by day only* (Fig. 1), necrotic lesions appeared but were retarded in relation to nontreated controls and had a reduced virus titre. These results support an earlier contention[7] that cells invaded by ring-spot virus by day resist necrosis more than cells infected at night. Both strains of mosaic virus, however, induced more prominent lesions (in all hosts) when synthesized by day.

Whereas, an external supply of $NO_3$ (50 p.p.m. of N) tended to reduce necrosis in Turkish tobacco leaves infected with ring spot it stimulated the necrotic action of yellow-mosaic virus in the same leaves, but reduced the amount of peripheral yellowing in the lesions. Such supplemental $NO_3$ under the same conditions did not reduce necrosis induced by the latter virus in the *N. tabacum–N. glutinosum* hybrid, but markedly reduced the amount of peripheral chlorosis. In this same hybrid, cyanide inhibited the synthesis of ring-spot protein, but in some instances a *secondary* stimulation in virus synthesis occurred. For example, in one experiment sectors were treated once with KCN for a period of 23 hours, starting 23 hours after inoculation. One hundred and forty-six hours after cessation of cyanide treatment the virus titre was determined. The extract from the cyanide-treated sectors produced 606 lesions as against only 93 for the controls. Such marked secondary stimulation of ring-spot synthesis was not observed in the varieties of *N. tabacum* and was not observed in these varieties with either strain of mosaic virus. The only observed prolonged alteration in the physiology of the hybrid tobacco leaves, following exposure to cyanide, was a marked reduction in the amount of brown pigment formed by oxidation (polyphenol dehydrogenase system) of tissues, which were killed by the virus *after cessation of cyanide treatments.*—MARK W. WOODS, Maryland Agricultural Experiment Station, College Park, Maryland.

*Erwinia carotovora, the Cause of a Soft Rot in Orchids, Cattleya* sp.—*Erwinia carotovora* (Jones) Holland apparently has not been reported attacking *Cattleya* sp. In September, 1940, two New Jersey orchid growers called the attention of J. W. Bulger[1] to a soft rot of cattleyas, obviously a pathological condition, and Dr. Bulger referred the material to the writers.

Infected parts show a dull water-soaked condition that spreads rapidly. The color is darker green than normal, and may become black when a break in the epidermis exposes the mesophyll to the action of air. Leaves, pseudobulbs, and rhizomes may be infected. In the leaf, wrinkling of the epidermis follows collapse of the tissue (Fig. 1). An exudate is often present, which becomes very dark brown when dry.

Tissue transfers from the margins of infected areas consistently yielded a white bacterium. Single-colony strains obtained by the dilution-plate method were made of the bacteria from both New Jersey sources. Inoculations of *Cattleya mossiae* and *Cattleya* sp. were made successfully in each of

[1] Bulger, J. W. Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

6 trials in which the host tissues were wounded by needle punctures. No infection resulted when the inoculum was applied on wound-free tissue or when the area inoculated was not covered by a film of water for 18 to 24 hours following inoculation. Reisolations were made and successfully used in inoculation.

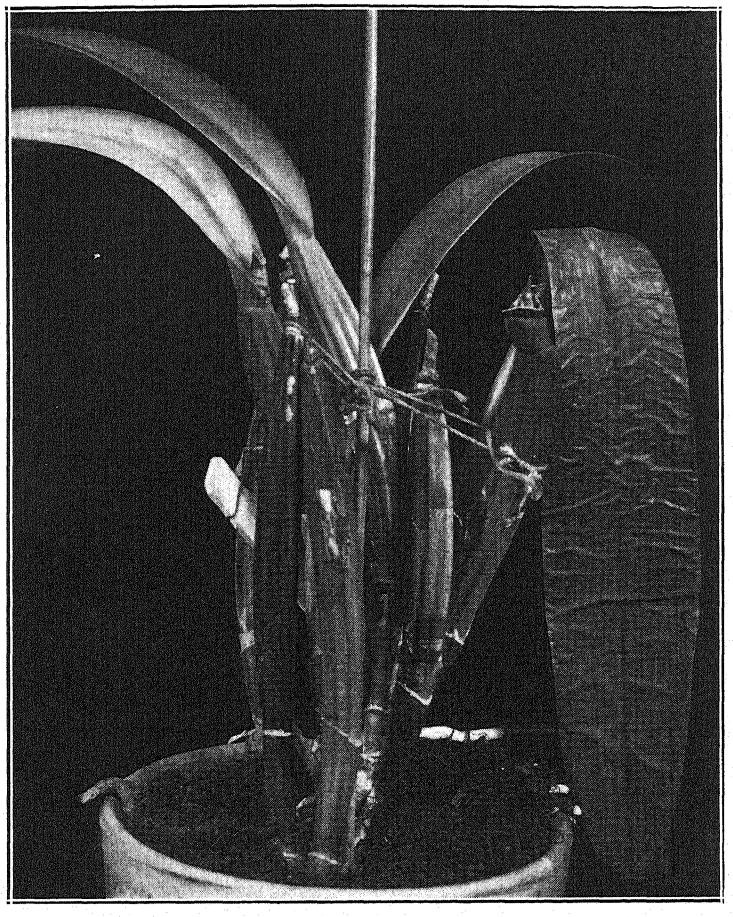


FIG. 1. Cattleya plant with inoculated leaf drooping and wrinkled. Photo by J. W. Bulger.

The bacteria from both sources were identical and are characterized as follows: Motile rods with peritrichiate flagella, forming white colonies, Gram-negative, no spores, gelatin liquefied, producing indol and hydrogen sulphide, starch hydrolyzed slightly, reducing nitrates to nitrites, forming acid and gas in dextrose, lactose, sucrose, and mannite, slight acid but no gas in glycerol, neither acid nor gas in maltose broth, and an acid curd in brom cresol purple milk.

Definite decay of inoculated leaves usually can be observed 24 hours after inoculation. Complete involvement of the leaf occurs in 4 to 7 days at room temperature. The bacteria pass readily from the leaf to the pseudobulb and into the rhizome, causing the death of the plant.

Matsumoto and Okabe[2] have reported *Erwinia carotovora* attacking *Phalaenopsis* sp. in Japan. They were successful also in inoculating *Cypripedium* and *Cymbidium*. In addition to these orchids, the writers have produced the disease on excised leaves of *Oncidium. Odontoglossum, Brassavola,* and *Lockhartia* by inoculation.

The disease is said to occur in "spots" on the benches, and has not been observed by the writers on plants not mechanically injured.—DONALD P. LIMBER and BERNARD A. FRIEDMAN, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Hoboken, N. J.

*A Leaf-spot of Hibiscus sp. Induced by Phytomonas syringae.*—Water-soaked spots (Fig. 1, A) on the leaves of hibiscus were observed in a coastal city of California. These spots were 1–10 mm. in diameter, brown to black, and of a water-soaked appearance. A yellowish-green zone or halo was sometimes observed surrounding the necrotic area. The spots developed during the cool, rainy weather of winter on plants growing near a house, under the drip of the eaves. With the coming of drier conditions, the disease ceased to spread.

Cultures from the large necrotic spots gave numerous colonies of bacteria in the dilution plates. Isolates from hibiscus yielded a white opalescent growth so closely resembling that of the organism causing black pit of lemons that inoculations were made through wounds in lemon fruits. Black pits (Fig. 1, D) promptly developed from these inoculations. The resulting lesions resembled those caused by the citrus blast and black-pit organism, *Phytomonas* (*Pseudomonas*) *syringae* (Van Hall). Smith and Fawcett,[1] and Smith[2] have shown that a number of different organisms described under different names and from different hosts will produce pits on lemon fruits, and are organisms closely related to, if not identical with, *Phytomonas syringae.* Rosen[3] has described a blast of roses induced by *P. syringae* that in his experiments infected lemon fruits.

Inoculations through injuries on the leaves of hibiscus by isolates for the hibiscus leaf spot and isolates for lemon black pits caused typical lesions (Fig. 1, B and C) to form in healthy leaves of hibiscus. Leaves of lilac inoculated with the hibiscus isolates also caused spots to develop typical of lilac blight.

A leaf spot on *Hibiscus* sp. has been described from Japan by Nakata and Takimoto[4] as caused by *Bacterium hibisci.* The Japanese disease is one that

[2] Matsumoto, T., and N. Okabe. On the causal organisms of the bacterial soft rot of Kotyô-ran, *Phalaenopsis aphrodite* Reichb. Jour. Soc. Trop. Agr. (Formosa) 3: 117–134. 1931.

[1] Smith, Clayton O., and Howard S. Fawcett. A comparative study of the citrus blast bacterium and some allied organisms. Jour. Agr. Res. [U.S.] 41: 233–246. 1930.

[2] Smith, Clayton O. *Pseudomonas prunicola* and *Bacterium citriputeale.* Phytopath. 21: 1091. 1931.

[3] Rosen, H. R. Rose blast induced by *Phytomonas syringae.* Jour. Agr. Res. [U.S.] 51: 235–243. 1935.

[4] Nakata, K., and S. Takimoto. Bacterial blight of hibiscus. Ann. Phytopath. Soc.

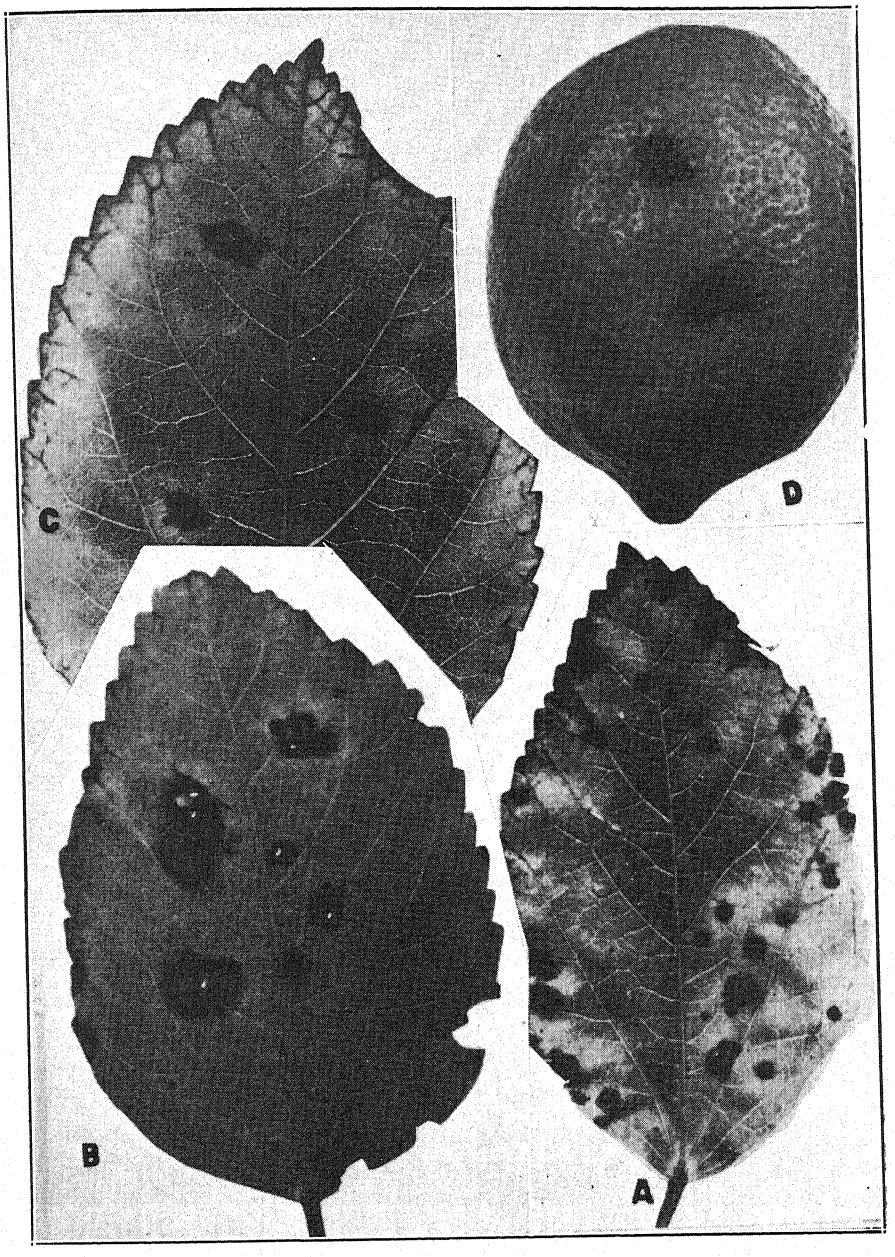



FIG. 1. A. Natural infection of hibiscus leaves from a coastal area. B. Spots from inoculations with an isolate from hibiscus through an injury on leaves of hibiscus, after 3 weeks. C. Spots induced in hibiscus with a culture of citrus blast isolated from a lemon fruit. D. Inoculations in lemon fruits with a culture isolated from hibiscus leaves, after 2 weeks.

attacked the cotyledons and leaves. The leaf symptoms of the Japanese and the California diseases agree in that the spots may have a halo. Inoculations by the Japanese scientists were successful only through injuries.

The morphological and cultural characteristics of *Bacterium hibisci* and *Phytomonas syringae*, as published by Bryan,[5] are very similar, the chief difference being in the absence of capsules in *Bact. hibisci*. The Japanese scientists did not report the coagulation of milk. (It has been reported by Smith and Fawcett that the coagulation of milk by *Phyt. syringae* is not always to be observed.) Lee[6] and Smith[7] have each described *Phyt. syringae* under different names as a new species. Smith did not report capsules, and Lee noted that no capsules or spores were observed. Bryan states that a small capsule could be demonstrated by Ribbert's dahlia stain. The hibiscus organism from California has a small capsule. A more critical comparative study of the California and Japanese organisms may show them to be the same, and that they are not different from *Phytomonas syringae*.—CLAYTON O. SMITH, University of California Citrus Experiment Station, Riverside, California.

Japan 1: 13–19. 1923. (In Japanese with English summary.) Also see Bot. Abst. 14: 862. 1923.

[5] Bryan, M. K. Lilac blight in the United States. Jour. Agr. Res. [U.S.] 36: 225–235. 1928.

[6] Lee, H. A. A new bacterial citrus disease. Jour. Agr. Res. [U. S.] 9: 1–8. 1917.

[7] Smith, Clayton O. Black pit of lemon. Phytopath. 3: 277–281. 1913.

---

The official 1942 ballots of The American Phytopathological Society, 582 in number, received by the Secretary, were opened and canvassed on December 26 by a special committee appointed by President Hutchins.

The following members received a majority vote for the respective offices:

President, one year—J. C. WALKER.

Vice-President, one year—J. J. CHRISTENSEN.

Councilor, two years—J. G. LEACH.

The Committee:

C. C. ALLISON
C. W. ELLETT
FOLKE JOHNSON
W. G. STOVER, *Chairman*

# BACTERIOLOGICAL STERILITY OF TISSUES DERIVED FROM SECONDARY CROWN-GALL TUMORS

ARMIN C. BRAUN AND PHILIP R. WHITE

(Accepted for publication May 27, 1942)

## INTRODUCTION

*Phytomonas tumefaciens* (Smith and Town.) Bergey *et al.*, upon inoculation into sunflower, Paris daisy, and probably other hosts as well, induces not only the production of primary tumors at the site of inoculation but also secondary tumors at points distant from the primary foci (2, 6, 9). These secondary tumors bear a superficial resemblance to the metastatic growths found in certain malignant animal neoplasia. The distribution of secondary growths, together with the morphological and cytological similarities of both primary and secondary crown-gall tumors to certain tumorous growths of animals and of man, has led to their frequent inclusion in discussions of neoplasia in general.

Erwin F. Smith, who studied the crown-gall problem intensively for more than two decades, believed that *Phytomonas tumefaciens* was intimately bound up with the host cells, multiplied as the host cells multiplied, and was directly responsible for the continued development of the tumor. Most other workers in the field have concurred in this view, although not always agreeing with Smith as to the exact position of the bacteria in the host tissue.

Although *Phytomonas tumefaciens* is known to initiate tumor formation in plants, this organism has not always been isolated from old primary tumors. It can seldom be obtained from secondary tumors in the sunflower even after special effort. This has led certain workers to question the necessity of the continued presence of bacteria for the continued proliferation of the host tissue and to postulate that the host cells involved may sometimes acquire a capacity for autonomous growth.

Studies on the question of autonomy of crown-gall cells have been complicated in the past by the fact that sterile tumor tissue was not readily available. Primary tumors usually contain, in addition to the causal agent of the disease, saprophytic organisms of many kinds. Recently, however, the writers of this paper have submitted evidence (8, 9) that bacteria-free tumor tissue can be secured from the nodular secondary tumors of sunflower plants, and that this tissue is capable of autonomous neoplastic growth both *in vivo* and *in vitro*.

Clones of tissue cultures isolated from 10 metastatic tumors were subjected to careful study. Although derived from different tumors, all showed similar morphological and cytological characteristics which differentiated them sharply from cambial or procambial tissue cultures isolated from healthy sunflower plants. The normal plant tissues grew in culture very

slowly, were usually green in color, and often developed roots. They showed considerable internal structural organization, evidencing a fairly pronounced response to the morphogenetic restraints characteristic of the same tissues when growing *in situ*. The tumor-tissue cultures, on the other hand, showed a high degree of independence. They grew very rapidly, were glistening white, and never developed roots. Internally they were composed mostly of parenchymatous tissue with scattered scalariform elements that never became organized into any coherent system. While cultures of healthy sunflower tissue increased in volume about 250 times in slightly more than a year, the tumor-tissue cultures showed a theoretical increase of a hundred million million times during the same period. Moreover, these tumor tissues retained undiminished their tumor-inducing capacity for more than a year. The tissue has been successfully transplanted from sunflower to the closely related Jerusalem artichoke and back again to sunflower plants, producing characteristic tumors in both hosts. The gross structure and histological picture presented by these transplant tumors were similar to those of the galls produced by *Phytomonas tumefaciens*. No tumors were ever produced when cultures of healthy tissues were similarly treated.

The cells of secondary crown-gall tumors behave, then, like malignant animal cells in several respects. They maintain an uncontrolled growth in the initial host. They can be transplanted successfully to other plants of the same or of closely related species without change in behavior. They retain these characteristics of uncontrolled autonomous growth, probably indefinitely, when grown both *in vivo* and *in vitro*.

It is the purpose of this paper to outline in detail the evidence upon which is based the conclusion that these autonomous and potentially malignant tissues are, in fact, free of *Phytomonas tumefaciens*.

## METHODS

Most of the present study is based on tissue cultures isolated from the nodular secondary tumors developed above the site of the primary tumor on sunflower plants (Fig. 1). Plants of sunflower (*Helianthus annuus* L. var. Giant Russian) were grown in compost soil in 4-inch pots and were kept on the greenhouse bench throughout the course of the experiments. When they had reached a height of 9 to 12 inches, they were inoculated with the highly virulent $A_6$ strain of *Phytomonas tumefaciens*. Inoculations were always made by needle puncture into an internode at a distance of not less than 3 inches from the apical bud. Primary tumors developed in all inoculated plants, followed in about 60 per cent of the cases by metastatic secondary tumors. It was felt that a thoroughgoing attempt to obtain bacteria from secondary-tumor tissues or evidence of the presence of bacteria in such tissues should be made.

When the nodular secondary tumors had grown to the size of a small pea, usually 5 to 6 weeks after inoculation, they were sacrificed. Tissue from such growths was isolated and grown *in vitro* according to methods described elsewhere.

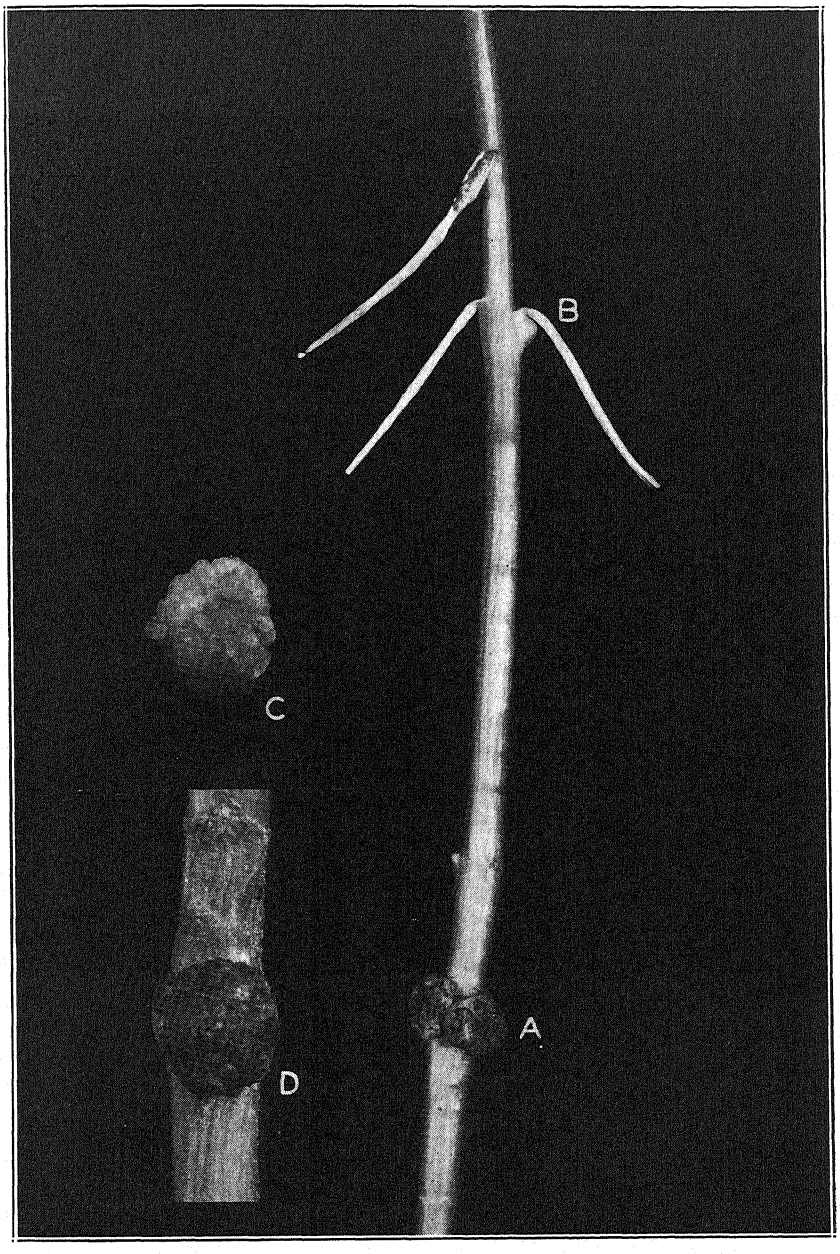



FIG. 1. A. Primary tumor formed at the point of inoculation in a sunflower plant. B. Nodular secondary tumor located 2 nodes above the primary tumor. The tissue composing this type of nodular overgrowth is generally free of bacteria. C. Bacteria-free tumor-tissue culture originally derived from a fragment of a secondary tumor similar to that shown at B. D. Tumor produced on a sunflower stem by the implantation and growth of a small fragment of a bacteria-free tumor-tissue culture. Note the similarity in structure between the bacteria-free tumor at D and the bacteria-containing tumor at A. (Photograph by J. A. Carlile.)

Bacteriological tests were applied to: (1) tissue freshly isolated from various regions of infected plants; (2) tissue cultures derived from one secondary tumor after 5, 10, 15, 20, and 25 passages *in vitro* and at various miscellaneous intervals between, tissue cultures derived from one additional tumor after 16 passages *in vitro,* and tissue cultures from 8 other secondary tumors after 9 passages *in vitro;* (3) tissue isolated from second-generation tumors produced by implanting tissue cultures in new hosts. These totaled about 1250 tests. Serological tests were applied to tissue cultures of the first group mentioned above after they had been maintained for 10 or more passages *in vitro.* These employed 125 cultures. About 1000 tissue cultures, mostly of this first group, were maintained for periods of 2 weeks or more growing on a nutrient suitable for the isolation of crown-gall bacteria. Grafting experiments in which about 700 tissue cultures were used constitute a 4th type of test the results of which bear on the question of the bacteriological sterility of secondary tumors and materials derived therefrom. Additional special tests also were made on a few tissue cultures and will be outlined later. In all, the results of a total somewhat more than 2000 tests of various kinds are included in the present paper.

## RESULTS

### Bacteriological Tests

Recovery of *Phytomonas tumefaciens* from certain types of tumor tissue is considered difficult, and the need for applying suitable methods when attempting to isolate this organism has frequently been emphasized. Crown-gall bacteria are considered present in pockets scattered throughout the tumor tissue. Success in isolation would therefore appear to depend in large measure on the method used to release the bacteria from these pockets. A thorough trituration of the tissue is obviously important. This was accomplished either by tearing with sterile needles, by grinding the material in a sterile mortar, or by crushing it in a sterile test tube with the aid of a glass rod. A few milliliters of nutrient-dextrose broth, potato-dextrose broth, or yeast-water-mannitol broth were added before crushing the tissues. The thoroughly triturated tissue was permitted to incubate at room temperature for periods ranging from 1 to 8 hours or longer. The tissue suspensions were then pipetted into sterile Petri plates and the desired agar media added. The plates were rotated in the usual manner to insure uniform distribution of the tissue. All plates were incubated at 25° C. for at least 3 weeks before being discarded.

*Bacterial Flora of Tissue Freshly Isolated from Various Regions of Infected Plants.* Fragments of tissue were excised aseptically from various regions of sunflower plants carrying well established infections of *Phytomonas tumefaciens.* These were triturated in media suitable for the isolation of this organism. Plates were incubated either until crown-gall organisms appeared or until they had remained sterile for at least 3 weeks. The results are shown in table 1. Primary tumors were 100 per cent positive. The

incidence of crown-gall bacteria decreased rapidly from the primary tumors upward so that, while secondary tumors at the first node above the primary were positive in 83 per cent of the 35 cases tested, tumors at higher sites were positive in only 4 per cent of the 181 cases tested. Apparently healthy tissues showed almost as high an incidence of infection, indicating that this 4 per cent might be looked upon as due to chance contaminants having no clear causal relation to the incidence of tumors. Secondary tumors at distances of more than one node from the primary were free of bacteria in by far the majority of cases.

This observation, although contrary to the usually accepted idea, is entirely consonant with Erwin F. Smith's observations. In his 1912 paper (7) he records, for plant No. 1 (Paris daisy), 20 plates from 3 secondary tumors and 21 plates from 3 "tumor strands" all negative. From plant VII he records an unspecified number of plates from 4 secondary tumors and 7

TABLE 1.—*Incidence of crown-gall bacteria in different regions of infected sunflower plants*

| | Primary tumors | Secondary tumors | | Apparently healthy tissues at a distance of 6–12 inches above the primary tumor | Total |
|---|---|---|---|---|---|
| | | Located at 1st node above primary tumor | Located at 2nd, 3rd, and 4th nodes above primary tumor | | |
| Number tested | 100 | 35 | 181 | 100 | 416 |
| *P. tumefaciens* isolated from | 100 | 29 | 7 | 2[a] | ..... |
| Per cent infected with *P. tumefaciens* | 100 | 83 | 4 | 2 | ..... |

[a] Each plate contained a single colony of *P. tumefaciens*.

tubes from 2 tumor strands, likewise all negative. From plant XIV he records an unspecified number of plates from a single ruptured tumor of which "a few" were positive. Specific tests from other plants were not recorded. Out of 13 secondary tumors and tumor strands tested, 12 thus appeared to be bacteria-free. While Smith attributed this to faulty technique and to the refractory nature of crown-gall bacteria, he did so apparently on the unproved assumption that tumefaction can occur only in the presence of bacteria. The actual observations are equally consonant with the alternative interpretation that the bacteria were not and had not been present in these regions.

*Bacterial Flora of Tissue Cultures from Secondary Tumors.* The methods used in isolating tissue for *in vitro* cultivation were similar to those employed for the isolation of crown-gall organisms except that the tissues were not triturated. Fragments of tissue were excised under aseptic precautions. No disinfectant of any kind was employed. The epidermis was first stripped off carefully. Tissue was then removed with a sharp scalpel

or a surgeon's ear curette. The fragments were placed on a semi-solid nutrient suitable for the growth of both excised plant tissues and crown-gall organisms. This consisted of White's glycine-thiamin-sucrose nutrient-salt solution to which 0.6 per cent of thoroughly leached agar was added. Fifty ml. of this nutrient was placed in 125-ml. Erlenmeyer flasks. Cultures that, in the first passage, developed contaminations either of *Phytomonas tumefaciens* or of air-borne or water-borne saprophytes (see above) were discarded, and only those that appeared sterile were used for further study. Cultures were divided at the end of each 2-week period until clones of the desired number of cultures were built up. Clones of tumor tissue were thus established from 10 secondary tumors. Eight clones have been carried for more than 9 passages, one clone for more than 16 passages, and another clone is now (May, 1942) in the 30th passage. Cultures have remained for a minimum of 2 weeks in each passage in intimate contact, both at intact and at freshly cut surfaces, with a nutrient capable of supporting growth of crown-gall organisms. More than 1000 cultures have been so maintained. In all these tests, *P. tumefaciens has, after the first passage, never appeared on a single culture.*

The nutrient used, however, has a pH reading of about 5.5. This is below the optimum for the initiation of growth by very small numbers of the crown-gall organism. It was important, therefore, to extend these tests by the use of special methods better adapted to the detection of small numbers of organisms or of organisms in a semi-dormant state. A method for this work was developed as follows.

Pure cultures of *Phytomonas tumefaciens* were streaked on nutrient agar plates and incubated at 25° C. for 21 days, so as to bring them into a negative and, hence, semi-dormant growth phase approximating the condition postulated for the supposed bacteria in the tumor tissues. Organisms were then transferred from these aged cultures into sterile tap water and shaken vigorously to break up the bacterial clumps. Dilutions were made and varying numbers of bacteria were introduced into the various broth media to be tested. To simulate the effects that the presence of sunflower tissue might have on the initiation of bacterial growth, a small, finely ground, tumor-tissue culture was added to each tube of broth under aseptic conditions. For every tube inoculated with bacteria and tumor tissue, 2 tubes were inoculated with tumor tissue alone as controls.

In preliminary tests, tubes inoculated with tumor tissue alone never produced growth that could be identified as *Phytomonas tumefaciens.* Tubes of nutrient-dextrose broth plus 0.1 per cent agar containing tumor tissue to which bacteria in numbers of 10 or more were added regularly produced bacterial growth. This medium is therefore *suitable* for the initiation of growth from as few as 10 viable bacterial cells in the presence of sunflower tissue. It, therefore, was used in most subsequent experimental work. Potato-dextrose broth and yeast-water mannitol broth also were found to favor growth from small numbers of bacteria but were employed to a lesser extent.

TABLE 2.—*Occurrence of molds and bacteria in tissue cultures from secondary crown-gall tumors*

| Date | Tissue culture clone designation | Passage | No. of cultures | Medium used | Molds | Bacteria | Bacteria producing tumors on tomato plants | Percentage of cultures carrying crown gall |
|---|---|---|---|---|---|---|---|---|
| 5/30/41 | H 7 | 8 | 4 | Nut. dext. agar | 0 | 0 | 0 | 0 |
| " | H 13 | 5 | 4 | Nut. dext. agar | 0 | 0 | 0 | 0 |
| 6/6/41 | H 7 | 9 | 4 | Nut. dext. agar | 0 | 0 | 0 | 0 |
| 7/18/41 | Not recorded | – | 5 | Nut. dext. agar | 0 | 0 | 0 | 0 |
| " | " " | – | 5 | Pot. dext. agar | 0 | 0 | 0 | 0 |
| " | " " | – | 5 | Patel's medium | 0 | 0 | 0 | 0 |
| " | " " | – | 5 | Nut. dext. broth | 0 | 0 | 0 | 0 |
| " | H 7 | 10 | 24 | Pot. dext. agar | 10[a] | [a] | 0 | 0 |
| " | " | 10 | 76 | Nut. dext. agar | 8[a] | [a] | 0 | 0 |
| 9/26/41 | " | 15 | 33 | Nut. dext. agar | 1 | 6 | 0 | 0 |
| " | " | 15 | 33 | Pot. dext. agar | 0 | 2 | 0 | 0 |
| " | " | 15 | 34 | Patel's medium | 1 | 1 | 0 | 0 |
| 12/2/41 | " | 20 | 33 | Nut. dext. agar | 1 | 2 | 0 | 0 |
| " | " | 20 | 33 | Pot. dext. agar | 3 | 0 | 0 | 0 |
| " | " | 20 | 34 | Nut. dext. broth | 1 | 2 | 0 | 0 |
| 12/5/41 | H 13 | 16 | 14 | Nut. dext. agar | 0 | 0 | 0 | 0 |
| " | " | 9 | 11 | Pot. dext. agar | 0 | 0 | 0 | 0 |
| " | 1 | 9 | 5 | Nut. dext. agar | 1 | 0 | 0 | 0 |
| " | 2 A | 9 | 3 | Nut. dext. agar | 0 | 2 | 0 | 0 |
| " | " | 9 | 2 | Pot. dext. agar | 0 | 1 | 0 | 0 |
| " | 4 | 9 | 3 | Nut. dext. agar | 0 | 0 | 0 | 0 |
| " | " | 9 | 2 | Pot. dext. agar | 0 | 0 | 0 | 0 |
| " | 8 | 9 | 5 | Nut. dext. broth | 0 | 0 | 0 | 0 |
| " | 10 | 9 | 3 | Nut. dext. agar | 0 | 0 | 0 | 0 |
| " | | | 2 | Pot. dext. agar | 0 | 0 | 0 | 0 |
| " | 20 | 9 | 5 | Nut. dext. agar | 0 | 0 | 0 | 0 |
| " | 22 A | 9 | 5 | Nut. dext. agar | 4 | 1 | 0 | 0 |
| " | 22 B | 9 | 5 | Nut. dext. agar | 0 | 3 | 0 | 0 |
| " | H 7 | 13 | 314 | Nut. dext. agar | 3 | 32 | 0 | 0 |
| 2/27/42 | " | 25 | 96 | Yeast-water-mannitol agar | 6 | 0 | 0 | 0 |
| Total | ..... | ..... | 807 | ..... | 39 | 52 | 0 | 0 |

[a] Bacteria and molds were not distinguished in the record of this group.

In any bacteriological study contaminations do, of course, occasionally occur. Bacterial colonies occasionally appeared on plates poured from broth inoculated with tumor tissue. When this happened, colonies from such plates were always reinoculated into young sunflower or tomato plants, even when their appearance showed clearly that *Phytomonas tumefaciens* was not their major constituent. When crown-gall organisms are inoculated into these hosts, tumors are always produced so that such a test furnishes a positive means of identifying the bacteria if they are *P. tumefaciens*.

Tests on tumor-tissue cultures, using these methods, gave the results shown in table 2. Out of 807 cultures tested 91 were contaminated, 39 with molds and 52 with bacteria. All bacteria, however, proved to be secondary invaders not identifiable as *Phytomonas tumefaciens*, since in no case did they produce tumors when inoculated into young tomato plants. Six per cent of the tumor-tissue cultures tested were contaminated with bacteria. While this appears to be a relatively high percentage of contamination, it should be recalled that during trituration the tissues often remained exposed to the laboratory air for several minutes. We believe that in some instances, at least, contamination resulted during this period of exposure. This belief seems justified by the fact that frequently only one bacterial or mold colony appeared on the plates. In at least 3 instances, however, the tissues apparently became contaminated during cultivation in the tissue-culture flasks. On December 5, 1941, one group of old cultures of the H 7 clone used in most of this work was suspected, because of the slimy appearance of some of its members, of harboring bacteria. These cultures represented a group originally set aside for some special experiments and left in the flasks without being transferred for 4 to 6 weeks instead of being transferred at 2-week intervals. The 314 cultures comprising this group were, therefore, all tested bacteriologically. Thirty-two were found contaminated. Of these, all contained the same large, slow-growing, spore-forming bacterium. It appears likely that this organism had been introduced into a single culture at an earlier date and that it had become spread throughout this group during the division and transfer of the contaminated tissue. Similar results were obtained when the 2 A and 22 B clones, each consisting of 5 cultures, were tested.

Nutrient-dextrose, potato-dextrose, and yeast-water-mannitol broths containing 0.1 per cent agar and finely ground tumor tissues have been shown by us to permit the initiation of growth by very small numbers of bacterial cells. These broths were, therefore, used in still further checks on the presence of *Phytomonas tumefaciens* in the tissues. Thirty tumor-tissue cultures were ground separately in sterile mortars, each with a few ml. of the desired broth. The tissue suspensions were then pipetted under aseptic conditions into test tubes containing either nutrient-dextrose, potato-dextrose, potato-dextrose plus 1 per cent yeast water, or yeast-water mannitol broths. Some of the tubes of each set were so slanted as to expose a large surface of the liquid to the air; others were kept upright and, consequently, had

only a small surface exposed. The results after a 6-week incubation period at 25° C. showed all but 3 of the 131 tubes sterile. One of the potato-dextrose-broth tubes was contaminated with mold, while 2 of the nutrient-dextrose-broth tubes were contaminated with a slow-growing rod-shape organism that formed long chains in the broth. These bacteria were inoculated to tomato plants, but no tumors resulted. The plants were discarded after 4 weeks.

Since the conventional methods of isolating *Phytomonas tumefaciens* failed to demonstrate the presence of this organism in the tumor-tissue cultures, still another method was tried. Ten tumor-tissue cultures were cut into sections about 100 μ in thickness. These tissue slices were aseptically transferred to solidified nutrient-dextrose agar and potato-dextrose agar in Petri plates. Bacteria-containing primary-tumor tissues from sunflower plants were similarly treated. The 50 slices of primary-tumor tissues yielded cultures of the crown-gall organism in every instance, while all of the more than 200 slices of the secondary-tumor tissue cultures remained sterile. These sterile tissue slices, in many instances, grew quite well on the potato-dextrose agar, and after a period of one month typical masses of tumor tissue were found.

*Bacterial Flora of Second Generation Galls Produced on Sunflowers by Implantation of Tumor-tissue Cultures.* One of the procedures used in studying the autonomy of tissue cultures has been the implantation of pieces of tissue under the bark of young healthy sunflower plants, either as small masses containing viable tumor cells or as triturated débris. Since the bringing of bacteria or bacteria-containing tissue in contact with a wounded susceptible surface of a suitable host plant regularly results in the production of a tumor, every graft of this sort that *fails* to produce a tumor constitutes evidence of the bacterial sterility of the tissue used. The converse is, however, *not* true, as we have shown elsewhere (10). Out of a total of about 800 such grafts carried out in the past year, about 650 have produced no tumors, showing that the tissues used were free of demonstrable tumefacient agents and, hence, free of viable crown-gall organisms.

Tumors are, as we have shown elsewhere, produced in a considerable percentage of cases upon implantation of tumor-tissue cultures. Tissue taken from such a second-generation tumor was ground in beef-extract peptone-dextrose broth, incubated for 2 hours, and then plated (5 plates) on beef-extract peptone-dextrose agar. One plate developed a spreader type of bacterial colony (not *Phytomonas tumefaciens*). The other 4 remained sterile. Eleven isolations of tumor tissue were made from a second old and partly necrotic second-generation tumor and placed on White's glycine-thiamin agar nutrient. Six developed bacterial contaminants, but none of these produced tumors on inoculation into tomato plants.

These results, although meager, are consistent with the rest in demonstrating that second-generation tumors as well as most secondary tumors and all tissue cultures are free of crown-gall bacteria by all bacteriological methods used.

## Serological Tests

Since bacteriological tests by several methods failed to reveal crown-gall organisms in tumor-tissue cultures derived from secondary tumors, serological tests were applied as a further check on the presence of the crown-gall bacteria in these tissues. Rabbits weighing about 2000 g. were used. All were bled at the beginning of the experiment and their sera shown to be free of agglutinins for the strain of *Phytomonas tumefaciens* used in this work. Two rabbits were injected at spaced intervals with tumor tissues that had been maintained *in vitro* for at least 15 passages. As checks, 2 additional rabbits were injected with primary-tumor tissue that was about one month old and was known to contain the crown-gall organism. The antigens used for injection in both cases were prepared by thoroughly grinding 3 g. of tumor tissue together with sterile saline (0.85 per cent NaCl) in a mortar. The suspensions were then filtered through a 60-mesh wire sieve and injected intraperitoneally in 5-cc. doses, except for the first injection in which 2 cc. were used. Twelve injections were given at 4-day intervals and the rabbits were bled 8 days after the last injection. Trial bleedings revealed that the maximum titre against the crown-gall organism in the bacteria-containing tumor tissue was reached after the 9th injection, no increase in titre being observed during the last 3 injections. A saline suspension of a 36-hour nutrient dextrose agar culture was used as the test antigen. Agglutination tests showed that the sera of rabbits injected with the bacteria-containing primary tumor tissues had titres against the $A_6$ strain of *P. tumefaciens* of 1–180 and 1–320, respectively. The other two sera, prepared by injecting ground-up tumor-tissue cultures into rabbits, did not react against the crown-gall organism even at serum dilutions as low as 1 : 1. Complement-fixation tests in which formolized bacillary suspensions of the $A_6$ strain of *P. tumefaciens* were used as test antigens fully confirmed the results of the agglutination tests.

## Special Tests

*The Question of Filterable Forms.* The possibility that some morphological variant of *Phytomonas tumefaciens* other than the normal rod-shape organism may have been present in the tissues must also be considered. Levine (5), working with 3-month-old cultures of this organism, observed amorphous masses of protoplasm similar to the symplasm described by other workers. He also found in these cultures minute spherical bodies, faintly-staining cocci, and slender filamentous elements. These observations, he believed, supported the idea of there being a life cycle in these bacteria. D'Herelle and Peyre (4) suggested that a filterable probacterial form, rather than the normal rod-shape organism, might actually be the true cause of the crown-gall disease. They reported that filtered extracts from sugar-beet galls were capable of inducing tumors on healthy beets. The normal rod-shape organism was, however, always isolated from the latter. Almon and Baldwin (1), working with the closely related organisms, *Rhizobium trifolii*

Dangeard and *R. leguminosarum* Frank, found filterable forms present in a large majority of cultures that had aged for periods of one month or longer. Agglutination tests indicated some group relationship between the aberrant types and the normal forms. The aberrant types that developed from the filterable forms were, however, noninfectious on their respective host plants. Other reports recently published indicate that filterable forms may be present in other bacterial species.

Perhaps the most widely used and the most satisfactory method thus far developed for the cultivation of filterable forms is the Hauduroy technique (3). A modification of this method was used. Three tissue cultures from secondary tumors carried through 10 passages *in vitro* were ground separately, each in 5 ml. of sterile nutrient broth. The tubes were permitted to incubate at room temperature for one month. No visible bacterial growth appeared in any of the tubes. Three additional tumor-tissue cultures were then ground in a similar manner and were incubated at room temperature for 48 hours. These likewise remained sterile. Portions consisting of 0.5 ml. of broth from each of the 3 tubes that had been incubated for one month were added separately to 3 flasks containing yeast-water-mannitol agar, potato-dextrose agar, and nutrient-dextrose agar. The second series of tubes, which contained tumor-tissue cultures ground and incubated for 48 hours, were treated in a similar manner. The flasks were incubated at 25° C. for one week, after which time fresh portions of 0.5 ml. of broth were added to each of the flasks and the washings transferred to fresh agar of the same composition as that used for the original flasks. Twelve successive passages on this nutrient were made in 5 of the 6 cases. One of the flasks containing the potato-dextrose agar became contaminated with mold during the 5th washing. At the end of the 12th passage, none of the 5 remaining flasks showed any visible evidence of bacterial growth. Microscopic examination of the clear washings failed to reveal the presence of any recognizable bacterial elements. The results presented make it unlikely, therefore, that any filterable or otherwise aberrant form of the crown-gall organism was present in, and in any way a significant factor in the development of, the secondary tumor tissues grown *in vitro*.

## Plant Inoculation Studies

Plant-inoculation tests were made as an additional check on the presence of the normal or aberrant forms of *Phytomonas tumefaciens,* as well as on the presence of other agents that might be involved in inciting the continued abnormal proliferation of the cells of tumor-tissue cultures. Two methods of introducing the tissues into the host plants were used. The first of these consisted of grinding the tumor-tissue culture and placing the crushed material in wounds in the stems of sunflower plants. The wounds were tightly bound with sterilastic tape in a manner similar to that used when fragments of tumor-tissue cultures were grafted in the host (9). Out of 200 tests made in this way, all except one healed without significant deviation from normal

wound response. In this one instance a small slow-growing tumor developed. After it had reached the size of a pea, the tumor was crushed in the usual manner and tested for its possible bacterial content by plating in Patel's medium. *P. tumefaciens* was not isolated and the plates were discarded at the end of 4 weeks. That the tumor did not arise from a chance contamination with *P. tumefaciens* is indicated by the localized growth, which is in marked contrast to the diffuse tumefactions that arise when large wounds are thus contaminated. It is believed probable that a small fragment of the tumor tissue had not been sufficiently crushed to destroy all viable tumor cells and that the subsequent growth of these residual cells resulted in the formation of the small tumor.

The second method of introducing the tumor-tissue cultures into the host plant involved the thorough grinding of the tissues in a sterile mortar together with a small amount of nutrient-dextrose broth. After an incubation period ranging from a few minutes to 8 hours, the entire tissue suspension was introduced into either sunflower or tomato plants by means of needle punctures. Approximately 100 separate punctures were necessary to use up each tumor suspension. After inoculation the plants were placed in a moist chamber for 18 hours. This was done to prevent the drying out of the droplets of broth and thus permit the multiplication of any bacteria that might be present in the triturated tissues. A total of 32 tumor-tissue cultures were tested, involving more than 3000 separate inoculations. In no case did proliferation of the host tissue result. Galls were invariably produced when bacteria-containing primary-tumor tissue was similarly tested.

The possibility remained, however, that some inhibitory substance might be present in the tumor-tissue cultures that prevented small numbers of bacterial cells from establishing themselves in the host plants. To test this, tumor-tissue cultures were ground up in the usual manner and a few ml. of a dilute suspension of crown-gall bacteria (approximately 1000 organisms per ml.) were added. The tissue suspension together with the bacteria was introduced into tomato and sunflower plants by means of needle punctures. Approximately 50 bacterial cells were inoculated together with a small amount of ground tumor tissue into each puncture. Typical galls were obtained in every instance. It is, therefore, obvious that the ground tumor-tissue cultures did not have any deleterious influence on the gall-stimulating ability of *Phytomonas tumefaciens*.

The presence of some agent in the tumor tissues capable of causing continued abnormal stimulation of the cells, but not itself able to initiate tumor formation, had still to be considered. It is well known that *Phytomonas tumefaciens* may, under certain conditions, become attenuated naturally and that a decrease in virulence also can be brought about by transferring the organism successively through 15 to 20 passages in a medium containing glycine. Both naturally attenuated and glycine-attenuated sister-cell cultures of the $A_6$ strain of the crown-gall organism were introduced into tomato and sunflower plants, together with thoroughly ground tumor-tissue culture

suspensions. Both of these attenuated cultures were capable in themselves of producing cellular proliferation in the hosts in excess of the normal wound response, but they never formed typical large tumors in their host plants. The plants inoculated with the mixture of a crushed tumor suspension and an attenuated culture showed no more response than did the check plants treated with the attenuated cultures alone. These results show, there-

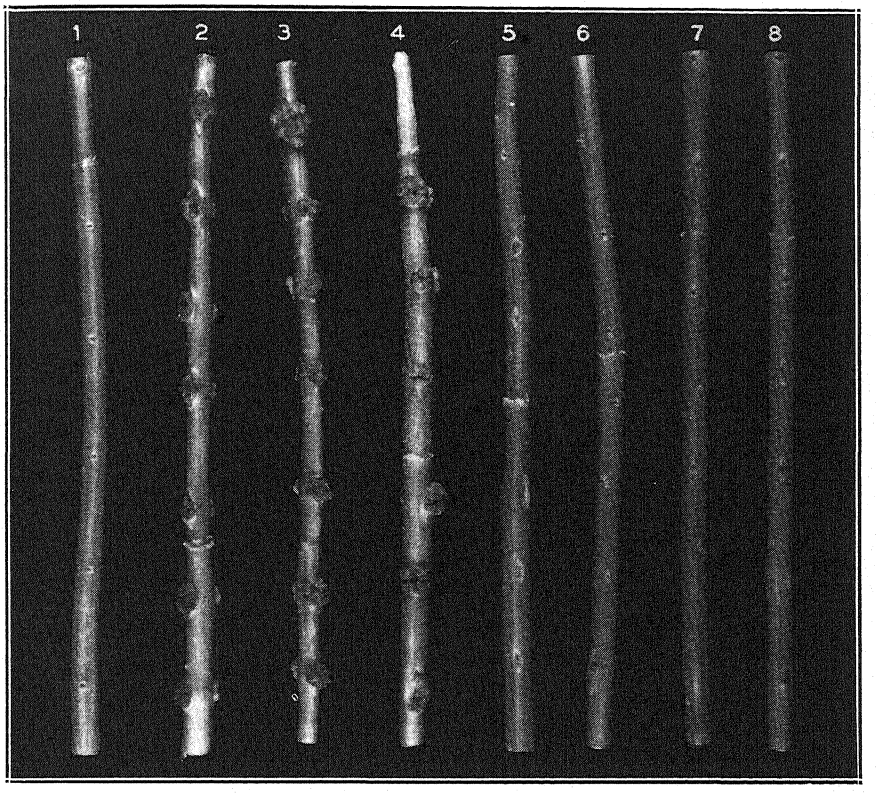



FIG. 2. Summary of plant inoculation studies. Sunflower plants were inoculated by means of the needle-puncture method with: 1, a thoroughly ground tumor-tissue culture; 2, bacteria-containing primary-tumor tissue similarly treated; 3, finely ground tumor-tissue culture plus a dilute suspension of a virulent culture of *Phytomonas tumefaciens;* 4, a virulent culture. Note tumor formation in 2, 3, and 4 where the crown-gall organism was known to be present. 5 and 7 were inoculated, respectively, with a naturally attenuated and a glycine-attenuated culture of the crown-gall organism. In both instances slight proliferation of the host tissue resulted in excess of the normal wound reaction. 6 and 8 were inoculated with the respective attenuated cultures plus a small thoroughly triturated tumor-tissue culture. These results show that there was no demonstrable agent present in the tumor-tissue cultures that could bring about the continued abnormal stimulation of the host cells after tumor formation had been initiated by the attenuated cultures. (Photograph by J. A. Carlile.)

fore, that there was no demonstrable agent present in the tumor-tissue cultures that could bring about the continued abnormal proliferation of the cells after tumor formation had been initiated by attenuated cultures of the crown-gall organism. The results of the plant-inoculation studies are summarized in figure 2.

To ascertain whether some tumefacient agent might be present in or carried by this crown-gall organism, filtrates of virulent cultures were examined. The virulent strains employed were either lysed with specific bacteriophage or were incubated in sterile saline for several weeks to encourage autolysis. The lysed suspensions were filtered through sintered glass bacteriological filters (porosity "65 on 3") or through Berkefeld N candles. The filtrates were introduced into tomato or sunflower plants either alone or in company with a partly attenuated culture of *Phytomonas tumefaciens*. In no case did cellular proliferation result in excess of that found in the check plants. All of these tests indicate that neither filterable bacterial forms nor recognizable viruses were present in tumor-tissue cultures after their prolonged cultivation *in vitro*.

## DISCUSSION

The most distinctive characteristic of malignant cells is their independence of the influences that govern the hereditary form of the organism, either animal or plant, in which they occur. All normal cells are subject to a rigid control mechanism when growing *in vivo*.

In work reported elsewhere it has been shown by the writers that the tumor cells in crown gall of plants behave like true malignant animal cells in that they show a greatly increased proliferative power over normal cells, both *in vivo* and *in vitro*, and at the same time retain their tumor-inducing capacity probably indefinitely when grown in tissue cultures or when transplanted in series to suitable host plants.

Speculations as to the origin of malignant cells have been numerous, and these have extended over a period of many years. Two general working hypotheses are now current, intended to explain how malignancy originates. The first of these postulates is that some foreign agent, intimately bound up with the cells, stimulates their growth and multiplication. The second postulate is that normal cells have themselves been modified into new cell types or mutants that are no longer subject to the control mechanisms of the host.

Tumor formation in crown-gall is known to be initiated by a bacterial parasite, *Phytomonas tumefaciens*, and it has generally been assumed that this organism is directly responsible for the continued multiplication of the cells. According to this concept, then, crown-gall tumors would logically fall, like the virus-induced tumors of animals, into the first category listed above, namely, that in which malignancy is attributed to the presence of a foreign agent.

The results of the present investigation have demonstrated, however, that, although *Phytomonas tumefaciens* induces tumor formation, its presence is not always required for the continued rapid proliferation of the cells. This conclusion is based on results of experiments designed not only to eliminate, insofar as the methods now available permit, the possibility of the normal or of aberrant forms of the crown-gall organism being present in the tissues, but also to consider the possibility of a virus that might either be trans-

mitted by or act in association with the bacteria in stimulating cell multiplication. More than 2000 tests designed to demonstrate such agents, bacterial or otherwise, have all given negative results.

The possibility that a growth-promoting chemical might have been introduced into the cells at the time they were first actively stimulated and that this substance, even though continually used up during the multiplication of the cells, might still be present in sufficient quantity to cause them to behave abnormally has not been entirely eliminated. The crown-gall tumor tissue has now been carried in artificial culture for 30 transfers involving a period of more than one year and has increased in theoretical volume more than a hundred million million times; yet, this tissue will have to be carried through at least 13 more generations before the theoretical limits (the equivalent of $\frac{1}{2}$ molecule of $H_2O$ per cell) are reached. However, since the tumor tissues have continued to increase at an *undiminished* rate through 30 generations *in vitro* and have at the same time retained their tumor-inducing capacity undiminished, it seems very unlikely that any such chemical might still be a determining factor in this abnormal behavior.

It seems necessary, therefore, to fall back on the second hypothesis, that a permanent alteration in the cells has taken place. The fact that the tumor cells when cultivated for long periods of time *in vitro* retain their peculiar cultural and cytological characteristics as well as their tumor-inducing capacity, in the absence of any demonstrable stimulating agent, strongly suggests that these are permanently altered cells that reproduce true to type and against which there is no control mechanism in the host. It is clear that, although the original stimulus to abnormal proliferation must have come in some way, either directly or indirectly, from the crown-gall organism, the continuation of this behavior becomes at an early stage quite independent of this original stimulus. It becomes a characteristic of the tumor cells themselves. What the nature of this fundamental change in the character of the cell is we cannot say and it seems needless to speculate at this time. It is believed, however, that the bacteria-free crown-gall tumor cells possess many of the essential characteristics of true malignant animal cells and represent, therefore, a potentially useful material with which to study certain basic principles involved in the etiology of malignancy.

## SUMMARY

The bacteriological sterility of tissues derived from secondary crown-gall tumors has been examined.

The results of more than 2000 tests of various kinds are included in the present paper. Viable tumor-tissue cultures have failed to yield cultures of *Phytomonas tumefaciens* in a single instance, when grown on nutrients suitable for the isolation of this organism, when thoroughly crushed and incubated in suitable nutrients, or when cut in thin slices and incubated on media known to favor the growth of this organism.

Special tests suitable for the isolation of small numbers of bacteria or of dormant phases, filterable forms, viruses, or noncultivable forms have likewise consistently yielded negative results.

Implantation of such tumor-tissue cultures in sunflowers and artichoke plants gave rise to typical crown-gall tumors that again failed to yield crown-gall organisms when subjected to the usual tests.

Tumor-tissue cultures, when used as antigens, failed to reveal any crown-gall organisms either by agglutination or complement-fixation tests.

It is believed that the evidence presented provides unequivocal grounds for concluding that the tumor-tissue cultures isolated from secondary tumors are entirely free of *Phytomonas tumefaciens* or of other recognizable tumor-inciting agents.

These bacteria-free crown-gall tumor cells possess many of the essential characteristics of true malignant animal cells, and represent, therefore, a potentially useful material with which to study certain basic principles involved in the etiology of malignancy.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

LITERATURE CITED

1. ALMON, L., and I. L. BALDWIN. The stability of cultures of *Rhizobium*. Jour. Bact. 26: 29–50. 1933.
2. BRAUN, A. C. Development of secondary tumors and tumor strands in crown gall of sunflowers. Phytopath. 31: 135–149. 1941.
3. HAUDUROY, P. Les ultravirus et les formes filtrantes des microbes, 392 pp. Masson et Cie. (Paris). 1929.
4. D'HERELLE, F., and E. PEYRE. Contribution à l'étude des tumeurs expérimentales. Compt. Rend. Acad. Sci. 185: 227–230. 1927.
5. LEVINE, M. Morphological changes in *Bacterium tumefaciens*. Science 62: 424. 1925.
6. SMITH, E. F., N. A. BROWN, and L. MCCULLOCH. The structure and development of crown gall. U. S. Dept. Agr. Bur. Plant Ind. Bull. 255. 60 pp. 1912.
7. WHITE, P. R. Plant tissue cultures. Biol. Rev. 16: 34–48. 1941.
8. ———, and A. C. BRAUN. Crown-gall production by bacteria-free tumor tissues. Science 94: 239–241. 1941.
9. ———, and ———. A cancerous neoplasm of plants. Autonomous bacteria-free crown-gall tissue. Cancer Res. 2: 597–617. 1942.

# PERONOSPORA TABACINA ADAM, THE ORGANISM CAUSING BLUE MOLD (DOWNY MILDEW) DISEASE OF TOBACCO

EDWARD E. CLAYTON AND JOHN A. STEVENSON[1]

(Accepted for publication June 2, 1942)

## INTRODUCTION

The fungus causing the blue-mold (downy mildew) disease of tobacco belongs to the Peronosporaceae, which includes a number of important crop-plant pathogens, all of which are obligate parasites. Blue mold is the type of disease that commands attention, and its sudden appearance in the United States as a disease of cultivated tobacco presented students of the fungi with a number of problems. Some of these have concerned:

(1) Present status of identification methods for the tobacco downy mildew and other Peronosporaceae.

(2) Source of the infection in the United States.

(3) Reason for the disappearance of the disease following the 1921 outbreak.

Question 1 involves the broad problem of identification in an important group of plant parasites, and hence being of general interest, will be considered in greatest detail. Questions 2 and 3 are of less general interest, and the writers merely wish to present certain material that leads them to conclusions different in some respects from those recently advanced by Wolf (20) and Stevens and Ayres (17).

## THE IDENTIFICATION OF PERONOSPORA TABACINA BY CONIDIOPHORE AND CONIDIA MEASUREMENTS

Students of the Peronosporaceae have used extensively length and breadth measurements of conidia in describing and identifying species. Most descriptions are based on material collected on a single host plant, which, in the case of *Peronospora tabacina* Adam, was *Nicotiana tabacum*. However, *P. tabacina* attacks many other species of *Nicotiana*, and a question that naturally arises is whether spores collected on other host species would be comparable in size with those collected on *N. tabacum*. To obtain definite information, the following experiment was conducted.

Thirty collections of blue-mold leaf material were gathered from 12 different species of *Nicotiana*, all grown in a single greenhouse during the period October, 1940, to April, 1941. There was no outside source of inoculum during this time, so all collections may be assumed to be the same fungus, more particularly because separate strains of the blue-mold organism are unknown. Each different collection from the same species

[1] The writers acknowledge the efficient assistance of Alice J. Watson, formerly of the Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture.

of *Nicotiana* (Table 1) was taken at a different time and from a different crop of plants.

To provide adequate "grand mean" values with which to compare the previous collections, 40 lots of blue-mold-infected leaves were obtained from *Nicotiana tabacum*. These collections were made over the same period, October, 1940, to April, 1941. All collections were preserved alike, and spore measurements were made by one person for reasons set forth by Harter (13). For each collection, 10 measurements were made of conidiophore length, and 25 each of conidia length and breadth. All were searched carefully for oospores, and, where found, 10 measurements were taken of the outside diameter. The objective was to determine whether by spore measurements made under the most favorable conditions it would be possible to prove that a downy mildew collection on *N. attenuata*, for example, was the same as the downy mildew of cultivated tobacco. As a test of whether the spore sizes recorded did or did not show significant differences, the writers have subjected the entire mass of data to statistical analysis. The formula for measuring significance was

$$t \sqrt{\frac{\text{Error } M^2(N_1 + N_2)}{N_1 \times N_2}}$$

Analysis of the data shows that despite the fact that conidiophore length varied considerably within collections, and hence a rather large difference was required for significance, 23 out of the 30 collections differed significantly in size from the grand mean, and all but one of the differences were significant at the 1 per cent level. Thus, the species collection measurements tended to be different from the *Nicotiana tabacum* grand mean, and hence the measurements did not provide a positive basis for identification. Furthermore, the differences were not consistent. Thus one collection on *N. stocktoni* had longer conidiophores and one, shorter conidiophores than the grand mean.

Conidia length and breadth has been the generally preferred method for identifying *Peronospora* species. However, considering length and breadth, 25 of the 30 collections were significantly different in one or both of these dimensions; and, again, most of the differences were significant at the 1 per cent level. Hence, it must be concluded that the conidia dimensions of the *Nicotiana* species test collections tend to differ from the *N. tabacum* grand mean.

Further analysis of the data in table 1 has shown (a) that the differences between the 40 mean values for *Nicotiana tabacum* collections were highly significant, and (b) that the variability was still greater between the 30 mean values for the *Nicotiana* collections. Hence the production of conidia on different host plants increased the amount of size variability. Variation *within samples* was substantially the same regardless of host plants.

It has been suggested (22) that a reason for the variability in pub-

TABLE 1.—*Measurements of Peronospora tabacina conidiophores and conidia*

| Collection number | Host plant *Nicotiana* | Conidiophores—length | | Conidia—length | | Conidia—breadth | |
|---|---|---|---|---|---|---|---|
| | | Range μ | Mean μ | Range μ | Mean μ | Range μ | Mean μ |
| 21 | *attenuata* | 321–465 | 380.1[b] | 16–22 | 18.44 | 13–19 | 15.42[b] |
| 31 | " | 225–366 | 300.1[c] | 15–22 | 18.56 | 11–17 | 15.04 |
| 14 | *benthamiana* | 264–420 | 346.5[c] | 13–21 | 18.56 | 12–19 | 15.00 |
| 8 | " | 180–300 | 246.9[c] | 15–20 | 17.24[c] | 12–17 | 14.84 |
| 9 | " | 225–330 | 277.2[c] | 14–21 | 18.16[b] | 13–18 | 15.08 |
| 13 | *quadrivalvis*[a] | 324–540 | 432.3 | 14–21 | 16.96[c] | 12–16 | 13.80[c] |
| 33 | *caesia* | 480–780 | 632.1[c] | 16–24 | 18.32[b] | 11–17 | 13.04[c] |
| 15 | *glauca* | 312–511 | 412.8 | 15–21 | 17.28[c] | 12–17 | 14.56 |
| 23 | " | 450–762 | 584.1[c] | 15–22 | 17.88[c] | 12–16 | 13.88[c] |
| 30 | " | 510–954 | 721.8[c] | 16–21 | 18.04[c] | 12–18 | 14.32 |
| 32 | " | 363–615 | 471.6 | 15–23 | 18.52 | 12–16 | 13.64[c] |
| 38 | " | 409–996 | 624.1[c] | 15–21 | 17.84[c] | 12–15 | 13.92[c] |
| 51 | *gossei* | 270–348 | 303.9[c] | 15–22 | 18.36 | 12–17 | 14.56 |
| 22 | " | 276–423 | 337.8[c] | 16–22 | 18.28[b] | 12–19 | 14.52 |
| 5 | *nesophila* | 315–420 | 364.4[c] | 12–24 | 16.96[c] | 12–18 | 13.72[c] |
| 11 | " | 213–309 | 249.9[c] | 13–21 | 17.60[c] | 12–17 | 14.60 |
| 24 | " | 306–705 | 546.6[c] | 15–21 | 17.92[c] | 12–16 | 14.36 |
| 28 | " | 249–360 | 309.1[c] | 16–21 | 18.36 | 11–17 | 15.12 |
| 39 | " | 240–336 | 294.6[c] | 16–22 | 18.60 | 13–19 | 15.40[b] |
| 42 | " | 252–354 | 310.5[c] | 15–20 | 17.80[c] | 13–16 | 15.12 |
| 10 | *repanda* | 345–600 | 463.5 | 15–24 | 19.52 | 13–19 | 15.36 |
| 72 | " | 414–621 | 514.8[c] | 15–23 | 18.64 | 11–19 | 15.90[c] |
| 34 | " | 405–681 | 572.7[c] | 16–21 | 18.20[b] | 12–15 | 14.32 |
| 37 | *stocktoni* | 336–477 | 415.8 | 13–19 | 16.92[c] | 12–16 | 13.92[c] |
| 26 | " | 279–330 | 307.3[c] | 16–21 | 18.96 | 13–19 | 15.72[c] |
| 41 | " | 309–783 | 549.0[c] | 15–20 | 17.88[c] | 10–16 | 13.64[c] |
| 16 | *sylvestris* | 405–825 | 589.5[c] | 15–24 | 18.08[c] | 12–16 | 14.08[c] |
| 29 | " | 321–456 | 393.3 | 15–23 | 19.20 | 11–18 | 14.16[b] |
| 17 | *tomentosa* | 270–540 | 366.5[c] | 14–21 | 17.12[c] | 12–16 | 13.32[c] |
| 27 | *wigandioides* | 318–570 | 456.6 | 15–21 | 18.08[c] | 12–17 | 14.04[c] |
| | *N. tabacum grand mean* derived from 40 collections | 330.4–552.48 | 433.9 | 16.0–23.33 | 19.13 | 12.58–17.75 | 14.83 |
| | Significant difference between mean of any sample and grand mean of 40 samples of *N. tabacum* (calculation based on individual values from all 70 collections): | | | | | | |
| | at 5 per cent level | 50.4 | | 0.78 | | 0.54 | |
| | at 1 per cent level | 66.3 | | 1.02 | | 0.71 | |

[a] Received as *N. bigelovii* var. *quadrivalvis*.
[b] Significantly higher or lower than average of 40 samples of *N. tabacum* at 5 per cent level.
[c] Significantly higher or lower than average of 40 samples of *N. tabacum* at 1 per cent level.

TABLE 2.—*Measurements of Peronospora tabacina conidia reported by previous investigators*

| Report by | Conidia | | | |
|---|---|---|---|---|
| | Length | | Breadth | |
| | Range | Mean | Range | Mean |
| | μ | μ | μ | μ |
| (1) Adam | 16–29 | 22 | 13–19 | 17 |
| (2) Angell and Hill | ..... | 18–28 | ..... | 14–17 |
| (3) Armstrong and Albert | ..... | 17.9–19.5 | ..... | 13.1–15.4 |
| (10) Godoy and Coste[a] | 15–24.5 | 21 | 12–16 | 14.5 |
| (21) Wolf *et al.*[a] | 15–28 | ..... | 12–18 | ..... |
| (22) Wolf *et al.* | 10.5–24 | 18.4 | 10.5–22 | 15 |

[a] The name *P. nicotianae* was used.

lished reports on *Peronospora tabacina* spore size might be that too few measurements were made. Thus if a person made enough measurements they would reach a true value. However, table 1 shows that with means based on only 25 measurements the difference required for significance was only about 5 per cent. This is very low, and proves that merely increasing the number of measurement within collections would not solve the problem. If, under the relatively uniform conditions provided, conidia measurement failed as a means for *P. tabacina* identification, what would be expected under more variable conditions?

To answer this question, the data reported by previous investigators are given in table 2.

TABLE 3.—*Conidia size of Peronospora species as reported by Berlese and Gäumann*

| Peronospora Group | Peronospora Species | Host plant Genus | Conidia length | | Conidia breadth | |
|---|---|---|---|---|---|---|
| | | | Range[a] | Mean[b] | Range[a] | Mean[b] |
| | | | μ | μ | μ | μ |
| Leiothecae | | *Dipsacus* | | | | |
| | | *Knautia* | | | | |
| | | *Scabiosa* | | | | |
| | *P. violaceae* | *Succisa* | 30–47 | 35 | 20–26 | 19 |
| | *P. phyteumatis* | *Phyteuma* | 20–26 | 21 | 14–17 | 14 |
| | *P. urticae* | *Urtica* | 20–27 | 30 | 18–22 | 22 |
| | *P. valerianellae* | *Valerianella* | 23–28 | 25 | 16–20 | 19 |
| | | *Papaver* | | | | |
| | *P. arborescens* | *Meconopsis* | 20–24 | 16 | 16–20 | 15 |
| | *P. grisea* | *Veronica* | 22–27 | 24 | 16–20 | 16 |
| | *P. chrysosplenii* | *Chrysosplenium* | 20–25 | 24 | 16–19 | 20 |
| | *P. lamii* | *Lamium* | 18–24 | 27 | 15–20 | 21 |
| | *P. candida* | *Anagallis* | 22–26 | 19 | 16–20 | 16 |
| | *P. antirrhini* | *Antirrhinum* | 25–27 | 25 | 15–17 | 21 |
| | *P. ficariae* | *Ranunculus* | 20–29 | 27 | 15–20 | 22 |
| Calothecae | | | | | | |
| | *P. calotheca* | *Asperula* | 20–23 | 26 | 12–17 | 16 |
| | *P. myosotidis* | *Myosotis* | 16–24 | 21 | 12–15 | 16 |

[a] Conidia ranges from Berlese.
[b] Conidia means from Gäumann.

The data in table 2 show a maximum range in conidia length of 10.5 to 29 μ with mean values from 17.9 to 28 μ, and a maximum range in breadth from 10.5 to 22 μ with mean from 13.1 to 17 μ. The spread is very much greater than in table 1, which could be attributed to more variable environmental conditions, and the fact that the material was handled differently and the measurements made by different persons. Considering the range of values in both tables 1 and 2, it appears reasonably certain that *Peronospora tabacina* could not be definitely identified by conidia measurements, and it is of interest to know whether the situation with *P. tabacina* is the exception or the rule.

To test this point, 13 species were selected at random from the classical monographs of Berlese (5) and Gäumann (9), the former giving conidia size in terms of range, the latter in terms of the mean. Table 3 lists the two sets of measurements. It will be noted that in 10 of the 13 species Gäumann's

TABLE 4.—*Measurements of Peronospora tabacina oospores*

| Collection No. | Diameter | |
|---|---|---|
| | Range | Mean |
| | μ | μ |
| 43 | 27–39 | 32.9 |
| 44 | 30–38 | 34.5 |
| 45 | 33–40 | 37.5 |
| 46 | 30–39 | 34.2 |
| 48 | 33–45 | 39.9 |
| 50 | 27–34 | 30.6 |
| 51 | 30–36 | 32.4 |
| 65 | 24–31 | 27.8 |
| 70 | 27–36 | 32.7 |

Significant difference between any 2 means at 5 per cent level ..... 2.9 μ
Significant difference between any 2 means at 1 per cent level ..... 3.9 μ

measurements lie completely outside the range given by Berlese. For only 3 species, do the two sets of measurements correspond. This appears to be good evidence that Peronospora conidia measurements quite generally tend to differ, and a large amount of other corroboratory data could be cited.

### OOSPORES AS AN AID TO THE IDENTIFICATION OF P. TABACINA

Out of the 70 collections studied, oospores were found in 9, and all of these were *Nicotiana tabacum*. Thus, it is evident at the start that under ordinary circumstances comparatively few collections may be expected to show oospores. Angell and Hill (2) stated that of hundreds of collections made in Australia, only one had oospores. The measurements of the oospores in each of our 9 collections are given in table 4.

Table 4 figures indicate a great variability in the size of *Peronospora tabacina* oospores. Computations show that many of the differences between mean values were highly significant. Thus, collections 48 and 50, made simultaneously in the same area, have a mean difference of 9.3 μ, and the

difference required for significance at the 1 per cent level was only 3.9 μ. Collection 48 was the largest, and collection 65 the smallest, and as between these two, 48 was 37 per cent greater in minimum diameter and 45 per cent greater in maximum diameter. Furthermore, the ranges of oospore size of different collections may not even overlap. Thus, the range of collection 65 was 24 to 31 μ, and for collection 48, 33 to 45 μ. Despite the variability of the figures in table 4, however, it might reasonably be expected that they do not represent a maximum of variability, because they were all collected in one season in one general area and handled in all respects alike. Table 5 lists the oospore measurements for *P. tabacina* that have been previously published.

The variability represented by the *Peronospora tabacina* oospore measurements reported in the literature and collected in table 5 is rather amazing. The range as reported by Wolf alone is 24 to 75 μ, and in Australia the combined measurements of Adam and Angell and Hill provide a range of

TABLE 5.—*Measurements of Peronospora tabacina oospores reported by previous investigators*

| Report by | Diameter | |
|---|---|---|
| | Range | Mean |
| | μ | μ |
| (1) Adam | 35–60 | 46 |
| (2) Angell and Hill | 28–50 | ..... |
| (7) Clayton and Stevenson | ..... | 32–40 |
| (21) Wolf *et al.*, 1934[a] | 45–75 | ..... |
| (22) Wolf *et al.*, 1936 | 24–43 | 32 |

[a] The name *Peronospora nicotianae* was used.

28 to 60 μ. All the oospore measurements for the Peronosporaceae as listed by Gäumann and Berlese provide a range for the entire genus of only 22 to 60 μ, so that it is evident that for *P. tabacina* the reported dimensions as given by highly responsible workers cover somewhat more than the accepted range for the genus. Furthermore, the minimum mean size—32 μ—as reported by Clayton and Stevenson (7), and Wolf *et al.* (22) is only about 70 per cent of the general mean of 46 μ reported by Adam (1) in his original description of *P. tabacina*. It seems apparent that *P. tabacina* oospores may be considered as not distinctive in appearance and extremely variable in size.

## THE STATUS OF P. NICOTIANAE

In 1898, Spegazzini (16) described as *Peronospora nicotianae* a downy mildew that he collected on a native *Nicotiana* in Argentina. This species has been considered distinct from *P. tabacina* by Adam (1) and others on the basis of an apparent difference in size of conidiophores and conidia. These supposed differences in size do not exist, however, as the writers have pointed out previously (7), but there still remain two features of the Spegazzini description that do not fit the blue-mold fungus found in the

United States. First, Spegazzini stated that his conidia germinated indirectly, to form zoospores, and second he described as oospores bodies, ranging in size from 50 to 80 μ, that are very different in appearance from the oospores found associated with the blue-mold fungus in Australia and the United States.

Prior to 1935, no Nicotiana downy mildew had been reported from South America since the Spegazzini report of 1898. Within the past 5 years, however, Wolf (20) has reported receiving *N. tabacum* material with typical

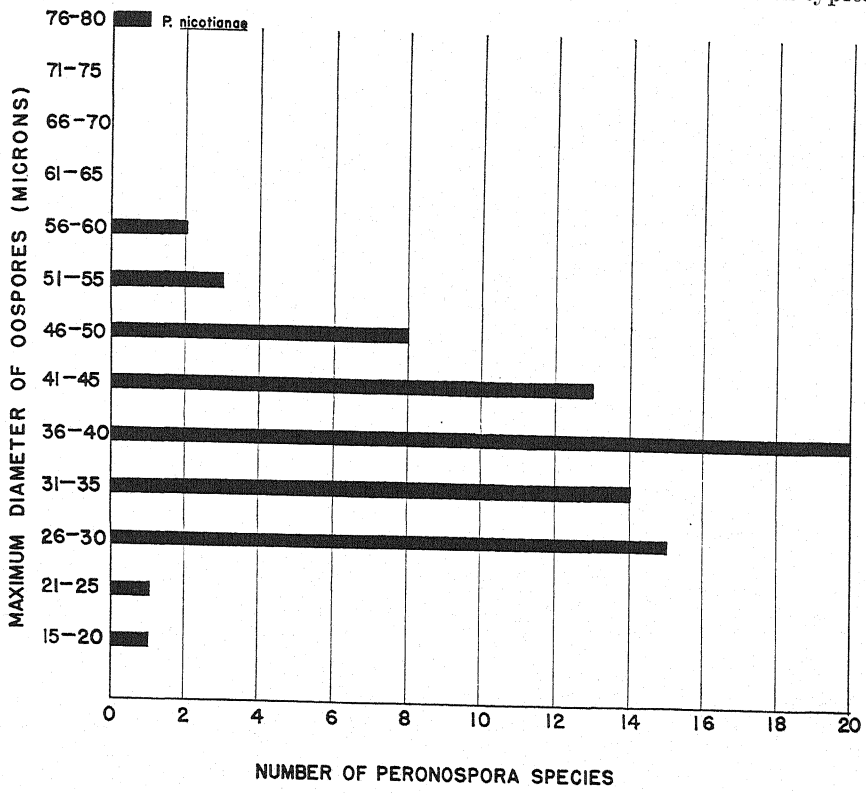



FIG. 1. The range of oospore size in the genus *Peronospora*.

*Peronospora tabacina* oospores from Rio Grande do Sul, that portion of Brazil nearest Argentina; and the writers have received similar material from the same area, which did not, however, show oospores. Still more recently, Godoy and Coste (10) reported a downy mildew of cultivated tobacco in Argentina. Thus, it is now established that a downy mildew, similar to that in the United States and Australia, is a serious disease of cultivated tobacco in the South Brazil-North Argentina area. Godoy and Coste searched for the oospores reported by Spegazzini, but reported their failure to find any. They recorded their organism as *P. nicotianae* on the grounds that the conidia and conidiophore dimensions did not agree with *P. tabacina*. As table 2 shows, they really agree very well. The situation is,

consequently, that, since the Spegazzini report of 1898, no one has found the bodies he believed to be the oospores of his *Peronospora*. It has been suggested that these bodies appear to be typical *Peronospora* oospores. However, as figure 1 shows, a maximum size of 80 is completely outside the normal oospore size range of the genus; in fact, so far as the writers are aware, no one, excepting Wolf *et al.* (21), has reported *Peronospora* oospores in excess of 60 μ in diameter. Secondly, as *Peronospora* oospores they would fall in the group Calothecae, while *P. tabacina* is in the Leiothecae. Search through Gäumann shows that while Gäumann lists as many as 7 separate *Peronospora* species in one genus (*Ranunculus*), in no case does he report a single host genus attacked by *Peronospora* species that falls in the 2 separate groups. Thus, in the instance referred to, all 7 *Peronospora* species, parasitic on *Ranunculus*, were members of the Leiothecae. It appears, consequently: (1) that a fungus fitting the *P. nicotianae* description has not been observed since its original description in 1898; (2) that a fungus answering the *P. tabacina* description is very definitely present in the area from which *P. nicotianae* was reported; and (3) that the supposed oospores on which *P. nicotianae* is largely based are completely outside the known oospore size range for the Peronosporaceae. In view of these considerations, the existence of *P. nicotianae*, as described, is so seriously in doubt that it can be omitted from further consideration.

### THE STATUS OF PERONOSPORA TABACINA AS AN ENDEMIC OR INTRODUCED FUNGUS IN NORTH AND SOUTH AMERICA

Many years ago it was suggested by McAlpine (15) that *Peronospora tabacina* was probably endemic to Australia, and this view is now quite generally accepted. With respect to North America, Stevens and Ayres (17) have recently suggested that the evidence for endemism is very scant, and Wolf (20) regards *P. tabacina* as an introduced species in South America. A problem of this sort is not easy to settle conclusively, but the writers believe there is strong evidence in favor of the view that the fungus is endemic in North and South America, as well. In the first place, *P. tabacina* is an obligate parasite, dependent for its existence on the presence of suitable food plants, *i.e.*, members of the genus *Nicotiana*. It is very different, thus, from many fungi that can apparently exist for long periods as saprophytes, or which attack species scattered through many genera. It is true that *P. tabacina* attacks peppers and, to a very slight extent, tomatoes and egg plant (3). However, so far as known, these other species are only affected in areas where the primary infection could be derived from *Nicotiana*. Godfrey (11) recently collected downy mildew-affected pepper plants in Texas, and, on subsequent search, found heavily infected *Nicotiana repanda* in the near vicinity. The dependence of *P. tabacina* on *Nicotiana* host plants for food would limit endemism to areas having a native *Nicotiana* flora. These areas are North and South America and Australia. *P. tabacina* is, of course, a low-temperature organism, which would limit its occurrence

to temperate regions. The temperate-zone regions with native *Nicotianas* are (1) south Australia, (2) western United States, and (3) south Brazil and north Argentina. The early mycologists collected a *Peronospora* on the native *Nicotianas* in all these areas.

The first records of a tobacco downy mildew in the United States involved *Nicotiana glauca* and *N. bigelovii,* which table 1 shows are good host plants for *Peronospora tabacina.* It is interesting to note that tobacco growers in the United States were not troubled by a downy mildew from 1620 to 1921, due, apparently, to the fortunate circumstance that tobacco culture was confined to the East, and no native *Nicotianas* occur east of the Mississippi River. How the gap was finally bridged remains speculation.

It may be mentioned here that the tobacco cultivated by the Indians in the East prior to the arrival of white settlers was not *N. tabacum* but *N. rustica,* which is quite resistant to blue mold and, accordingly, does not enter into the present problem.

The view that *P. tabacina* is endemic to Australia, and was introduced into North and South America, the only other areas with a native *Nicotiana* flora, while such regions as New Zealand, South Africa, Europe, and Asia have all remained free from such an introduction, does not appear to be very plausible.

## THE DISAPPEARANCE OF TOBACCO BLUE MOLD IN 1921

The first appearance of this disease in 1921 caused much concern, and its failure to reappear the following year was a surprise. Stevens and Ayres (17) have recently discussed the theory that this failure to reappear might have been due to climatic conditions. After a study of available records, they, however, concluded that it would not appear that the presence of the disease was correlated with the weather. The writers have examined leaf material collected in 1921 and find no oospores present. Wolf *et al.* (22) have very clearly shown the dependence of the organism on oospores for overwintering. Hence, the suggestion is made that the fungus, in 1921, failed to form oospores and so died out. The area affected in 1921 was very limited. In recent years large areas have been repeatedly observed where oospores could not be found, and there was no indication of old-bed overwintering in these regions the following spring. At present, the area involved each year is so extensive that the chance of oospores not being formed is small. It is quite possible that oospores may live over several years. Certainly, the lack of success experienced by workers in attempting to germinate them would suggest such a possibility.

## DISCUSSION AND CONCLUSIONS

A study has been made of the value of conidiophore, conidia, and oospore measurements in the identification of *Peronospora tabacina.* Conidiophore length has been found to be both extremely variable and inconsistent, so that it has little or no taxonomic value. Results from conidia

measurements were more consistent, thus corroborating to this extent the conclusion of Gäumann that such measurements were the most suited to taxonomic purposes. However, it was conclusively proved that mean values for different collections of conidia tend to differ. Thus, when 30 collections were compared with grand mean values set up with the utmost care, the great majority of the 30 collection measurements were significantly different from the grand means. Consequently, the effort to establish a single mean value for the size of *P. tabacina* conidia does not have a sound basis. This view is supported by the findings of Angell and Hill (2), who reported that the size of conidia produced at different times of the year varied greatly, and Armstrong and Sumner (4), who found smaller but very significant differences for *P. tabacina* spores produced on different host plants. Analysis of the data in table 1 also showed that the variability in the mean values obtained from collections made from different *Nicotiana* species was much greater than where collections were made from a single host plant—*Nicotiana tabacum*. That variability in Peronospora conidia size is not limited to *P. tabacina* is strongly suggested by table 3, in which the spore ranges of Berlese and the means of Gäumann are compared for 13 species. It is apparent there that the correspondence between the 2 sets of values is generally poor. Also, workers have sought for years to decide whether *Peronospora hyoscyami* was the proper name to use for the tobacco organism as suggested by Farlow (8). On the basis of conidia size, it is possible to prove (1) they are the same size or (2) *P. hyoscyami* is larger, depending entirely on which of the various published measurements are used.

Taking *Peronospora tabacina* mean values for conidia size as given in tables 1 and 2, it is evident that mean length can range from 17 to 28 μ and mean breadth from 13 to 17 μ. However, such a range of mean values includes many other species of *Peronospora*, and hence conidia measurements cannot be relied upon definitely to identify *P. tabacina*. On the other hand, mean values above or below this range would be a strong indication that the fungus in question was not *P. tabacina*. Gäumann used oospore type to differentiate the genus *Peronospora* into 2 main groups—Leiothecae and Calothecae—but he concluded that the oospores were too similar in appearance, and too variable in size to be of help in species identification. Stevens and Ayres (17) recently have suggested that a definite identification of *P. tabacina* could be made only if the oospores were present. *P. tabacina* oospores however, have nothing to separate them from *P. hyoscyami* and dozens of other members of the Leiothecae group. They have an irregularly wrinkled wall without definite pattern. In table 4 the mean diameter values for 9 collections range from 27.8 to 39.9 μ—a variation of 43 per cent. Most workers have listed oospores size as ranges of individual values. Table 4 shows such a range for one collection to be 24 to 31 μ, and for another 33 to 45 μ. In table 5 the reports of *P. tabacina* oospore size by other investigators show a range 24 to 75 μ, while the range for the genus *Peronospora*, as given

by both Berlese and Gäumann, is only 22 to 60 μ. Thus, if anyone were to discover oospores associated with a downy mildew on *Nicotiana* that had measurements outside the published range for *P. tabacina,* they would be justified in concluding that the bodies under observation were probably not *Peronospora* oospores. From the diagnostic viewpoint, the great potential value of oospores would appear to be in providing material for pathogenicity studies. Up to the present, however, most workers have been unable to obtain germination.

The conclusion derived from the preceding is that *Peronospora tabacina* cannot be definitely identified on the basis of size or appearance of its conidiophores, conidia, or oospores. Furthermore, it is indicated that the same may hold true quite generally for the Peronosporaceae. However, while these fungi appear to be not sharply separated morphologically, they are very highly specialized with respect to parasitism. *P. tabacina* is practically limited to the genus *Nicotiana.* The decisive value of pathogenicity in species differentiation is well illustrated by the *P. hyoscyami* situation. The tobacco fungus went by this name from the time that Farlow (8) collected a downy mildew on *N. glauca* in California, which had conidia about the same size as *P. hyoscyami.* There was doubt as to whether the tobacco fungus was the same, but repeated sets of conidia measurements and the discovery of oospores of both species (1, 5) failed to settle the question. However, this problem was definitely settled when it was found quite recently (2) that the tobacco organism did not attack *Hyoscyamus niger.*

The Peronosporaceae as classified by Gäumann contain numerous separate species that are differentiated solely on the basis of conidia length and breadth. The writers do not believe that conidia size is sufficiently stable to make such differentiation valid, particularly in view of the fact that size values assigned to many species were based on few, and in some cases apparently a single collection. It appears that the true species situation can be settled only by extensive controlled cross-inoculation work. At present there is very little information of this sort available. In this connection, the recent findings of Hoerner (14) are suggestive. He demonstrated by inoculation experiments that the supposedly separate *Pseudoperonospora* species—*P. cannabina, P. celtidis, P. urticae,* and *P. humuli*—may be merely physiologic races of a single species.

A fairly complete study of the pathogenicity of *Peronospora tabacina* with respect to the various *Nicotiana* species has been completed and will be published. This should provide a definite basis for as thorough identification of *P. tabacina* as may be desired, and it should also make it possible to determine whether specialized races of the organism exist in the different regions affected.

At present there appear to be no grounds for belief that more than one *Peronospora* occurs on *Nicotiana.* Spegazzini's *P. nicotianae* has not been observed since it was described, and the supposed oospore bodies on which it is chiefly based are much too large (Fig. 1) to be those of *Peronospora.* If

*P. tabacina* is the only *Nicotiana* downy mildew, then the need for assuming that it has been introduced into South America disappears. The writers believe the evidence strongly favors the view that the tobacco fungus is native to North and South America and Australia.

It is suggested that a likely explanation of why blue mold disappeared after the 1921 outbreak in the Georgia-Florida area of the United States is that the fungus failed to produce oospores.

## SUMMARY

In considering the identification of *Peronospora tabacina*, it was found that mean values for length and breadth of conidia and length of conidiophores tend to differ significantly, and the true situation is better represented by a range of mean values, rather than a single mean.

Size variability was greater with collections made from different host plant species than with collections made from a single host plant species.

The range of means indicated for *Peronospora tabacina* conidia is 17 to 28 μ long by 13 to 17 μ broad. These ranges are too great, however, to permit definite identification by measurements.

The variations in oospore size are such that measurements could have little or no taxonomic value other than to indicate that the bodies in question were within or without the size range for the genus, which is approximately 20 to 60 μ.

Neither conidiophores, conidia, nor oospores have distinctive morphological characters that could aid species determination.

Evidence is offered to show that the condition prevalent with respect to the tobacco organism is representative of the general situation in the genus *Peronospora*, and that spore measurements generally have not provided a sound basis for species identification.

The only definite basis available appears to be pathogenicity, and the fungus causing blue mold of tobacco is practically limited in its parasitism to the genus *Nicotiana*.

There appears no reason to believe that more than one *Peronospora* attacking *Nicotiana* exists. The name accepted for this organism is *P. tabacina* Adam.

This fungus is believed native to all temperate zone regions having a native *Nicotiana* flora, *i.e.*, portions of North and South America and Australia.

BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

## LITERATURE CITED

1. ADAM, D. B. Blue mould of tobacco. Jour. Dept. Agr. Victoria **31**: 412–416. 1933.
2. ANGELL, H. R., and A. V. HILL. Downy mildew (blue mould) of tobacco in Australia. Council Sci. and Indus. Res. (Australia) Bull. **65**: 1–30. 1932.
3. ARMSTRONG, G. M., and W. B. ALBERT. Downy mildew of tobacco on pepper, tomato, and eggplant. Phytopath. **23**: 837–839. 1933.

4. ARMSTRONG, G. M., and C. B. SUMNER. Investigations on downy mildew of tobacco. S. C. Agr. Exp. Stat. Bull. 303: 1–23. 1935.
5. BERLESE, A. N. Saggio di una Monografia delle Peronosporaceae. 311 pp. (Portici). 1903.
6. BAKHTIN, V. [Note on the oospores of *Peronospora hyoscyami* deBary] (Mater. for Mycol. and Phytopath.) 5(2): 158–160. 1926. [In Russian. English rev. in Rev. Appl. Mycol. 7: 270. 1928.]
7. CLAYTON, E. E., and J. A. STEVENSON. Nomenclature of the tobacco downy mildew fungus. Phytopath. **25**: 516–521. 1935.
8. FARLOW, W. G. Notes on some injurious fungi of California. Bot. Gaz. **10**: 346–348. 1885.
9. GÄUMANN, E. A. Beiträge zu einer Monographie der Gattung, *Peronospora* Corda. 360 pp., 1923.
10. GODOY, E. F., and ALBERTO DELLE COSTE. El "mildew" del tabaco en la región tabacalera de Salta. Revista Argentina Agron. **7**: 221–227. 1940.
11. GODFREY, G. H. Noteworthy diseases of economic crops and native plants in lower Rio Grande Valley in spring of 1941. Pl. Dis. Rptr. **25**: 347–353. 1941.
12. HARKNESS, H. W. Fungi of the Pacific Coast. IV. Bull. Calif. Acad. Sci. **1**: 256–271. 1885.
13. HARTER, L. L. The personal element and light as factors in the study of the genus *Fusarium*. Jour. Agr. Res. [U.S.] **62**: 97–108. 1941.
14. HOERNER, G. R. The infection capabilities of hop downy mildew. Jour. Agr. Res. [U.S.] **61**: 331–334. 1940.
15. MCALPINE, D. Report by the Vegetable Pathologist in Ann. Rept. Dept. Agr. (Victoria) (**1899**): 222–269. 1900.
16. SPEGAZZINI, C. Sobre una nueva enfermedad del tabaco y el polvillo de la alfalfa. Oficina Química-Agric. Prov. Buenos Aires Bol. **4**: 1–14. 1898.
17. STEVENS, NEIL E., and JOHN C. AYRES. The history of tobacco downy mildew in the United States in relation to weather conditions. Phytopath. **30**: 684–688. 1940.
18. STEVENSON, J. A., and W. A. ARCHER. A contribution to the fungus flora of Nevada. Plant Dis. Rptr. **24**: 93–103. 1940.
19. WOLF, F. A. Status of investigations of tobacco downy mildew. Phytopath. **29**: 194–200. 1939.
20. ———. Downy mildew of tobacco in Brazil. Phytopath. **29**: 291. 1939.
21. ———, L. F. DIXON, RUTH A. MCLEAN, and F. R. DARKIS. Downy mildew of tobacco. Phytopath. **24**: 337–363. 1934.
22. ———, RUTH A. MCLEAN, and L. F. DIXON. Further studies on downy mildew of tobacco. Phytopath. **26**: 760–777. 1936.

# CERCOSPORA BLIGHT OF CARROT[1]

H. REX THOMAS[2]

(Accepted for publication May 30, 1942)

This study originated as a field investigation of carrot blight at Santa Maria, California. It is concerned primarily with the physiology of *Cercospora carotae* (Pass.) Solheim and with the host-pathogen relationships and epidemiology of Cercospora blight.

## PLANTS ATTACKED

*Cercospora carotae* is known to attack both wild and cultivated carrots (*Daucus carota* L., *Daucus carota* L. var. *sativa* DC.). Infection was obtained on *D. hispanicus* Gouan, *D. maritimus* Lam., *D. pulcherrimus* Koch ex DC., *D. maximus* Desf., *D. gingidium* L., *D. pusillus* Mich., and 107 carrot varieties and selections tested in the field for resistance to Cercospora blight. Although the species *D. pulcherrimus, D. pusillus, D. gingidium*, and *D. maximus* were less severely blighted, no important degree of resistance suitable for use in a breeding program was found.

## SYMPTOMS

Cercospora blight may attack any part of the leaf, but primary lesions usually are located along the edge of the leaflets, causing a lateral curling (Fig. 1, A). Such lesions are elongate, while those that are not marginal tend to be circular. A diffuse chlorotic border may surround the necrotic portion of the spot. The lesion first appears as a pin-point chlorotic area, which enlarges and soon develops a necrotic center. These small spots enlarge and coalesce until the whole leaflet is killed. During humid weather, the lower surface of the lesion usually appears light gray or silvery because of the characteristic mass of hyaline conidia. This is one of the most reliable macroscopic means of differentiating between Cercospora and Macrosporium blights. *Cercospora carotae* may sporulate abundantly on the surface of the blade or petiole before the host tissue is killed.

Linear blackish-gray lesions develop, which may cover the entire petiole of the older leaves (Fig. 1, B). These lesions, like those on the blade, develop a light gray or silvery cast when conidia are produced. Eventually the petiole may be girdled and the leaf killed.

[1] Joint contribution from the Division of Plant Pathology, University of California, Berkeley, and the Department of Botany, Purdue University Agricultural Experiment Station, Lafayette, Indiana, based on a thesis submitted to the Graduate School of the University of California, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Journal Paper No. 13, of the Purdue University Agricultural Experiment Station.

[2] The writer wishes to express thanks to Dr. M. W. Gardner and Dr. R. M. Caldwell for valuable suggestions and aid in preparing the manuscript, to Professor R. E. Smith for the organization of the project, to Dr. A. G. Newhall for his suggestions while located at Santa Maria, to the growers and shippers of Santa Maria Valley for supplying land and equipment, and to the Santa Barbara Agricultural Commissioner's Office for laboratory facilities while in Santa Maria.

STOMATAL PENETRATION

Stomatal penetration was studied both in stained sections of leaf tissue and in material imbedded in paraffin. Considerable difficulty was experienced in observing penetration, as less than 1 per cent of the germ tubes penetrated. All attempts to increase materially the percentage of invasions by exposing the plants to light, darkness, and various temperatures during the incubation were unsuccessful. Examination of several thousand germinating conidia revealed no evidence of direct penetration of the epidermis.

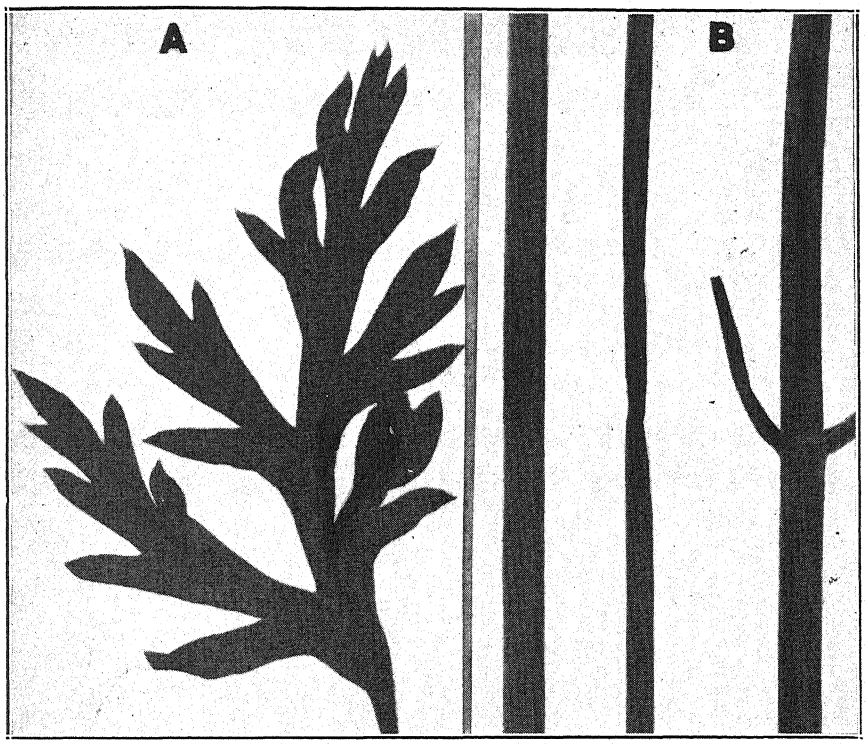



FIG. 1. A. *Cercospora carotae* lesion on portion of a carrot leaf. Note elongate marginal lesion and slight lateral curling of leaflet. B. *C. carotae* lesions on the petiole. Note linear shape of the lesion and silvery cast caused by the mass of conidia on the surface.

Some stomatal penetration occurred within 36 hours, but it was found most abundantly in material fixed after 48 to 72 hours. Stomata apparently had no attractive influence on germ tubes, as many of them grew across stomata without penetrating. Germ tubes appeared to grow at random until they accidentally came in contact with a stoma. No distortion of the somatal opening was caused during penetration, and no appresoria were found.

Entry through stomata is a phenomenon exhibited by other Cercospora species, such as *C. beticola* Sacc. (9) and *C. apii* Fresen (4), although wound infection is reported for *C. fabae* Fautrey (15).

DEVELOPMENT OF THE FUNGUS IN THE HOST TISSUE

The development of the mycelium inside the host was studied by examination of paraffin sections of leaves made 48, 72, 96, and 130 hours after inoculation with conidia. The penetrating hypha often enlarges after it has entered the stoma, plugging the stomatal cavity with globoid, thick-walled cells. The advancing hyphae usually invaded the mesophyll before advancing far laterally in the epidermis. Exceptions to this procedure were occasionally noticed when complete collapse of several epidermal cells would be found with no evidence of the fungus in the underlying tissues. Usually, such groups of cells were close to a point of stomatal infection. The advancing hyphae are intercellular at first, but intracellular invasion occurs promptly. Groups of collenchyma cells on the ridges of the petiole acted as a normal barrier. Very often mycelium would be abundant on both sides of one of these groups of collenchyma cells, but no hyphae were found in this tissue (Fig. 2). The fungus did not penetrate the petioles more than 5 or 6 cells in depth, growing chiefly in a lateral direction. In the leaf blade the fungus often completely permeated the mesophyll within 5 days.

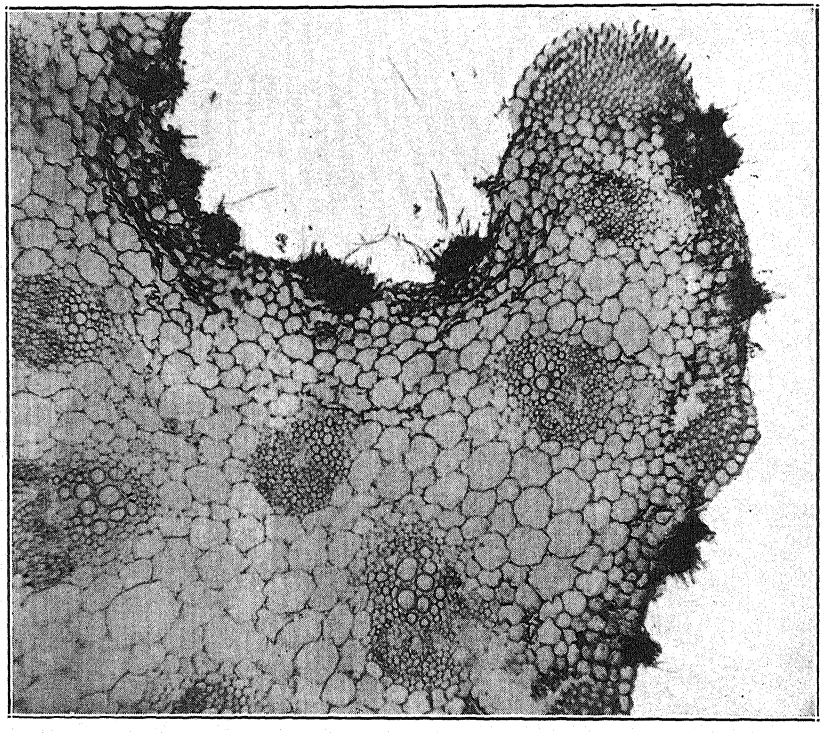


FIG. 2. Cross section of carrot petiole. Note that the fungus has not penetrated deeply and that conidiophore masses are found between groups of collenchyma. ×350.

SPORULATION

After the advancing hyphae have invaded to an extent of several cells,

vertically and laterally, the intertwined mass of substomatal hyphal cells sends up a tuft of hyphae that disrupts the stomatal opening and produces a fascicle of conidiophores 60 to 122 μ wide. On most of the petiole lesions a very compact fascicle of conidiophores was found as contrasted to the smaller and less compact conidiophore tufts found on both surfaces of the leaves. Occasionally, on the leaf, a single conidiophore was developed from a small group of cells in the stomatal cavity soon after infection. Each conidiophore produces several conidia in succession and 2 or 3 scars often are found near the tip of the conidiophore. The conidiophores, 2 to 3 μ in diameter, usually were not enlarged at the base. The conidia were cylindrical and hyaline to slightly colored. Among 100 conidia measured, there was a range in length between 40 and 110 μ with a mean of 95 ± 13.5 and a range in width between 2 and 2.5 μ with a mean of 2.2 ± 0.1. The number of septa varied from 1 to 6.

## CULTURAL CHARACTERS

Isolation of the fungus may be easily accomplished by making dilution platings of conidia on potato-dextrose agar. The conidia germinate within 12 hours on agar. The terminal cells usually germinate apically, the lower cells laterally. Very erratic germination occurred in hanging-drop preparations.

The fungus grows very slowly in culture, and it is 4 or 5 days before the growth from conidia on agar is macroscopically visible. The germ tubes and mycelium produced on agar are composed of short, globoid cells as contrasted to the long, narrow cells produced by conidia germinating in water. On agar a dark, dense, compact colony is formed on which conidia are usually present by the time the colony is visible. Cultures grown on artificial media for several months frequently lost their ability to produce numerous conidia. However, if a passage was made through carrots, such cultures produced abundant conidia.

The amount of growth and sporulation of the fungus on 8 kinds of media, adjusted to pH 6.2, was estimated in tube cultures kept at 22° C. for 3 weeks. For a more accurate measure, the rate of growth of hyphae from single conidia for 11 days after germination was determined microscopically. The most vigorous growth occurred on potato dextrose, but there also was good growth on pea, carrot-root, and prune agar. The poorest growth was made on malt and corn-meal agar. More conidia were produced on potato-dextrose, carrot, and carrot-leaf agar, than on pea, corn-meal, malt, and prune agar.

## EFFECT OF TEMPERATURE, HUMIDITY, AND pH ON THE GROWTH OF THE FUNGUS

The temperature relationships of the fungus were determined by estimating the amount of growth produced on potato-dextrose agar slants at temperatures of 3-degree intervals between 7° and 37° C. For more accurate measurements, the average maximum radial growth on agar plates of

hyphae from 10 conidia, after 11 days' exposure to the different temperatures, was microscopically measured. Most rapid growth occurred between 19° and 28° C., and there was almost no growth at 7° or 37° C.

The effect of temperature on the percentage germination of conidia was determined in a water suspension on glass slides covered with a thin layer of agar held for 24 hours in Petri-dish moist chambers at different temperatures. The maximum germination occurred between 16° and 28° C. and the least at 7° or 37° C.

The effect of temperature on sporulation was determined by placing 3 carrot petioles with young lesions at temperatures of 3-degree intervals between 7° and 37° C. After 24 hours it was found that abundant sporulation occurred between 13° and 28° C. No conidia were formed at 7° or 37° C.

Drops of a conidial suspension were placed on agar-coated slides and allowed to dry in air-tight, glass-stoppered bottles in which humidities ranging from 21.5 to 100 per cent were maintained by means of different concentrations of sulphuric acid. Although a few conidia germinated at 93.2 per cent humidity, no appreciable number germinated at less than 99 per cent. At humidities so close to 100 there is always a chance that condensation may have occurred. It was concluded that very little germination occurs unless a film of moisture is present. The conidia in the lower humidities were not injured because they always germinated satisfactorily when removed and exposed to moist conditions.

On agar slants the maximum amount of growth was made between pH 5.5 and 7.0. No growth occurred at pH 3 and growth declined markedly above pH 7.5. No effect on sporulation was observed, as the number of conidia appeared to be directly related to the size of the colony. The pH of the expressed juice of carrot leaves tested varied from 6.3 to 6.7.

### DISSEMINATION IN THE FIELD

Observations in the Santa Maria Valley indicate the probable importance of wind as an agent in the distribution of Cercospora blight inoculum. The prevailing wind is from the west and off the ocean. At harvest, plants along the east side of a field of infected carrots usually are more severely blighted than those on the west. Sufficient inoculum, apparently, is carried by the wind to increase infection on the east side. Also when there are fields of young carrots on the east and west sides of a field of older badly infected carrots, the former in the east field, next to the infected plants, are the more heavily infected.

During harvest, agar plates were exposed for 3 minutes at 10, 100, and 300 feet distant from the leeward side of a field badly affected with Cercospora leaf blight. The fungus was recovered from the air at all three locations.

Other workers (4, 8, 11, 14) have reported that wind is an important agent in disseminating the species of Cercospora attacking celery, peanuts, and beets.

SEED TRANSMISSION AND SEED TREATMENT

The seed umbels of carrot plants growing in an experimental plot at Santa Maria were found affected with Cercospora blight. Seeds saved from these plants had conidia of the fungus adhering to the seed coat. A few of the local growers at Santa Maria save a small quantity of seed for their own use. To determine if the locally-grown seed was contaminated with the fungus, portions of 8 seed samples were washed in 10 cc. of water and the liquid drained off and centrifuged. The precipitate and several cc. of liquid remaining in the bottom, after the supernatant liquid was poured off, were shaken up and sprayed on carrot leaves in the greenhouse at Berkeley. A few Cercospora leaf spots were found 14 days later on plants inoculated with 4 of the 8 samples. No infection was secured with the wash water from the seed harvested in the area where it is commercially saved nor on unsprayed controls. Repeated attempts to obtain infection from the centrifuged wash water from commercial seed were negative.

As very little locally grown seed is used within the Santa Maria Valley seed transmission is probably not an important source of infection. Although Cercospora is generally absent from the commercial seed fields near Sacramento, where a large part of the commercial seed is grown, the finding of Cercospora in one of these fields by Gardner, Kendrick, and Ark in June, 1940,[3] suggests that contaminated or infected seed occasionally may introduce carrot blight into territories previously free of the fungus. Doran and Guba (1) obtained no evidence that *Cercospora carotae* was carried on the seed. Other workers (4, 5, 6, 12, 14) have cited evidence indicating that seed transmission occurs with species of Cercospora affecting celery, beets, dill, soybeans, and peanuts.

Several treatments were applied to carrot seed to determine those that might be used without seed injury and would be effective both in controlling damping-off and freeing the seed of fungous and bacterial contaminants adhering to the surface. Seven replicates of 50 seeds from each of 14 treatments were sown in unsterilized soil in the greenhouse. The plots were arranged in a modified latin square. Stand counts were made 10 days after seeding. Compared with the stand of 33 per cent for the nontreated check the stands from seed treated with the following were significantly better: mercuric chloride (1–1000 for 5 min.), 49 per cent stand; cuprous oxide dust, 47 per cent; malachite green (1–2000 for 5 min.), 45 per cent; zinc oxide dust, 52 per cent; copper sulphate (2 oz. to 1 gal. for 1 hr.), 60 per cent; Spergon (dust), 60 per cent; Spergon (1–1200 for 5 min.), 45 per cent, and ethyl mercury tartrate (1 per cent preparation used as a dust), 55 per cent. Ethyl mercury phosphate (5 per cent preparation used as dust), 20 per cent; significantly decreased germination. Mercuric chloride (1–2000 for 5 min.), 41 per cent; mercuric chloride (1–3000 for 5 min.), 37 per cent; ethyl mercury tartrate (1–24,000), 35 per cent, and ethyl mercury phosphate (1–24,000), 33 per cent, did not significantly affect the percentage germination compared with the nontreated check.

[3] Letter from M. W. Gardner, dated October 17, 1940.

The effectiveness of the above treatments in freeing the seed from disease inoculum and other fungous or bacterial contaminants was determined by dipping carrot seed into a suspension of Cercospora conidia, drying the seed, treating, and then placing 25 seeds from each treatment on potato-dextrose agar plates. Four days later the seed treated with ethyl mercury phosphate and ethyl mercury tartrate, either as a liquid or a dust, mercuric chloride (1–1000) and Spergon (dust) were free from any fungous or bacterial growth. Dilutions of 1–2000 and 1–3000 of mercuric chloride sterilized 92 and 96 per cent of the seed, respectively. The other treatments were ineffective. It is possible that the active fungicidal ingredients in the treatments diffused into the agar and only inhibited the germination and growth of the contaminants adhering to the seed.

### PERSISTENCE IN SOIL

In the fall of 1937, Cercospora lesions were found on the first foliage leaves of 3-week-old carrot plants in several fields at Santa Maria, where blight had been serious on previous carrot crops. This suggested that infection came from the soil.

In July, 1938, soil samples were taken from the surface, 4 inches deep, and 6 inches deep in a field where the carrot crop harvested 2 months earlier was severely infected with Cercospora blight. These soil samples were placed in sterilized pots and planted to carrot seed in a greenhouse at Berkeley in which carrots had never been grown before. The pots were covered with a moist chamber, which was not removed until the young carrots were several inches high. No Cercospora infection was found on the plants. The pots were then filled with water and the surface of the soil stirred until the water was muddy. The tops of the plants were bent over and dipped into this muddy water. A moist cheese cloth cover was placed over the plants for 48 hours. At the end of 20 days, in the samples taken at the surface, 3 plants out of 1654 were found with sporulating Cercospora lesions. No infection was found on carrots planted in similar soil sterilized before use.

In contrast with certain other carrot-producing sections, there are carrots in the field at Santa Maria during the entire year. Often a crop is not harvested at maturity because of unfavorable market conditions. For example, carrots ready to harvest in December, 1936, were not harvested until February and March, 1937. Conidia of Cercospora were abundant at harvest on the older dead leaves of these plants and could provide a source of infection for the spring crop planted in nearby fields.

Infected carrot leaves were collected in December, 1936, and placed in wire containers. Some of these were placed on the surface of the soil, some 4 inches deep, some 6. These containers were removed in September, 1937, and typical Cercospora was secured on plants inoculated with water suspensions of the decayed leaves.

The possibility of the fungus establishing itself in the soil in the absence of a carrot crop was investigated. A suspension of Cercospora conidia was

placed in the center of Petri dishes filled with sterile soil. Upon examination later it was found that mycelium had developed and produced conidia. Similar results were obtained with unsterilized soil. Considerably more growth was made on soil high in organic matter than on soil of a sandy texture typical of that on which carrots are raised in the Santa Maria Valley.

Doran and Guba (1) have reported that *Cercospora carotae* hibernates in dead leaves of carrots in or on the soil; other workers (4, 7, 8) found that other parasitic members of this genus overwinter on the dead leaves of their hosts.

### RELATION OF TEMPERATURE AND HUMIDITY TO THE SEVERITY OF CERCOSPORA BLIGHT

Cercospora blight proved most serious at Santa Maria during June, July, and the first 2 weeks of August. Very little infection was found from the middle of August until March, when the disease began to appear generally in the valley. The damage caused early in the spring was slight, and it was June before any appreciable amount of infection developed.

During the summer and early fall very little rainfall normally occurs, but periods of very foggy weather are common. Carrot leaves are frequently covered with moisture from 6 p.m. until 8 a.m. There is little or no increase in the severity of Cercospora blight when the rainy season begins in the fall.

The factor or factors responsible for the decreased activity of Cercospora blight during late August and early fall have not definitely been determined. Hygrothermograph records (kept from August, 1936, to July, 1937) at Santa Maria show that during the period from April to July, when Cercospora was most active, the average minimum temperature fluctuated from 40° to 50° F. and the average maximum between 70° to 77° F. From July to November, the average minimum temperature ranged from 48° to 57° F. and the average maximum from 70° to 81° F. Laboratory tests showed that the fungus grew most rapidly on agar between 66° and 79° F

### THE EFFECT OF THE MINERAL NUTRITION OF THE CARROT PLANT UPON THE DEVELOPMENT OF CERCOSPORA BLIGHT

The effect of the mineral nutrition of the carrot plant upon the development of Cercospora blight was determined by counting the number and classifying the length of the leaf spots and by estimating the abundance of conidia produced on the leaf spots on the plants grown in solutions complete in all nutrients, or deficient in nitrogen, phosphorus, potassium, or calcium.

Three carrot plants of the variety Morses Bunching were grown in 6-inch, glazed pots, 5 pots for each of the 5 treatments. The pots were arranged in a latin square. A complete nutrient solution, made up according to Hoagland and Arnon's formula 1 (3) was supplied to the plants by a continuous drip mechanism, similar to that described by Pryor (10). When the plants had from 4 to 6 leaves, the pots were flushed with several liters of distilled water, and solutions deficient in nitrogen, potassium, phos-

phorus, or calcium, respectively, made up according to Hoagland and Arnon (3), were supplied to the plants. Extra carrot plants in each treatment were used to determine when deficiencies of nitrogen, phosphorus, and potassium occurred, by means of the plant tissue tests described by Thornton, Conner, and Fraser (13).

After deficiency symptoms were evident, the plants were sprayed with a suspension of Cercospora conidia from carrot leaves and placed in a moist chamber at 75° to 85° F. for 72 hours. Fifteen days after inoculation, the number of leaf spots per plant, length of leaf spots, and number and fresh weight of infected leaves were recorded. The approximate leaf area was determined by dividing the leaf weight by the weight per cm.$^2$ of leaf blade. The latter figure was determined by tracing around carrot leaves on paper, dividing the weight of the paper by the weight of one square centimeter of

TABLE 1.—*Influence of the mineral nutrition of the carrot on susceptibility to Cercospora carotae*

| Treatment | No.[a] of leaves | Area of leaf | No. of leaf spots per cm.$^2$ | Leaf spot length | Conidia production[b] | Weight of the fungus mat on leaf extracts[c] |
|---|---|---|---|---|---|---|
| | | *Cm.*$^2$ | | *Mm.* | *Class* | *Mg.* |
| Complete | 9.6 | 93.8 | 0.31 | 6.5 | 5 | 700 |
| −K | 7.1 | 33.3 | 0.28 | 4.7 | 4 | 750 |
| −P | 7.0 | 20.7 | 0.26 | 4.9 | 3 | 470 |
| −Ca | 5.9 | 44.6 | 0.19 | 2.7 | 2 | 270 |
| −N | 7.6 | 26.3 | 0.19 | 2.3 | 2 | 830 |
| Significant difference 5% level | 2.5 | 5.1 | 0.06 | 0.87 | ... | 210 |

[a] Composite figures for two experiments. An average of 10 plots, arranged in a latin square.

[b] Abundance of conidia produced on leaf spots on plants placed in humidity chamber for 36 hours. 0 = no conidia, 5 = many conidia.

[c] Growth of *Cercospora carotae* made in 60 days in a sterile leaf extract of plants grown in deficient solutions. Average of 5 flasks.

paper, and then dividing the weight of the leaves by this figure. The average number of leaves, average area per leaf, average number of leaf spots per cm.$^2$, average length of the leaf spots and abundance of conidia on the leaf spots are summarized in table 1. The data from the two experiments have been analyzed together by the analysis of variance method and only the composite results are presented.

## Potassium

Six days after being given the potassium-deficient solution, the potassium concentration of the leaf sap was low. However, it was approximately 10 days before potassium deficiency symptoms were evident. They consisted of a purplish tinge on the underside of the leaves and the development of new leaves that were smaller than those of plants on the complete solution. A deficiency of potassium did not significantly affect the number of leaf

spots but did significantly limit the length of the spots. The abundance of the conidia on the leaf spots as observed through the binocular microscope was less.

### Phosphorus

The first signs of phosphorus deficiency, which became evident after approximately eight days, were a stunting of the new growth and a purpling of the older leaves, particularly along the veins. The new leaves developed a darker green color. The number of leaf spots was not significantly affected, but the length of the leaf spot was significantly limited. The conidia were less abundant on the phosphorus-deficient plants than on either the complete-nutrient or potassium-deficient plants.

### Calcium

The characteristic symptoms of calcium deficiency were, in the first experiment, a stunting and chlorosis of the new leaves within 12 days after transference of the plants to the calcium-deficient solution, followed by a necrotic spotting of the leaves and tip necrosis five days later. At this point one millimol of calcium chloride was added to the solution to prevent further necrosis. In the second experiment the calcium chloride was added as soon as the stunting of the leaves was noticed to prevent development of necrotic symptoms. The number of leaf spots was significantly reduced. Approximately two-thirds as many were found as on the plants on the complete solution. The leaf spots were less than one-half as long, and the abundance of the conidia was approximately one-half that of the plants on complete nutrient.

### Nitrogen

Seven days after the plants were placed in the nitrogen-deficient solution the plant tissue test first indicated that the leaves were low in nitrate nitrogen. The new leaves were reduced in size, and both the new and old leaves were yellowish-green in color. The nitrogen-deficient plants were very similar to the calcium-deficient plants in behavior to infection. The number of leaf spots was only two-thirds that of the complete-nutrient plants. The leaf spots were only about one-half as long. The conidia were approximately one-half as abundant as on the complete-nutrient plants.

## GROWTH OF CERCOSPORA CAROTAE ON LEAF-JUICE EXTRACTS OF PLANTS GROWN IN VARIOUS MINERAL SOLUTIONS

The growth of the fungus on sterile juice extracts of plants grown under the different nutrient conditions was determined and compared with the development of the disease on such plants.

Carrot plants were transplanted into five 14-inch glazed pots filled with white silica sand and grown in a complete-nutrient solution until they had approximately ten leaves. The sand then was flushed with distilled water and the following solutions supplied, one to each pot: complete-nutrient,

potassium-deficient, phosphorus-deficient, calcium-deficient, and nitrogen-deficient.

When deficiency symptoms had developed, the leaves were ground, the juice extracted with a fruit press, and then diluted by one-third with distilled water. This mixture was sterilized by passage through a Seitz filter. Fifty cc. of the sterile extract was transferred aseptically to each of five 125-cc. Erlenmyer flasks. These were then inoculated with a suspension of conidia and stored at room temperatures for 2 months. *Cercospora carotae* grew very slowly in these extracts, and formed only a small mat in this period. The contents of each flask was filtered and the weight of the oven-dried fungus mat obtained.

There was significantly less growth made on the extracts from the phosphorus and calcium-deficient plants as compared with the complete-nutrient, potassium-deficient, and nitrogen-deficient plants. No correlation was found between the weight of the fungus mat on the extracts from the plant and the number of leaf spots, length of leaf spot, or number of conidia produced (Table 1).

## SUMMARY

*Cercospora carotae* attacks the leaf blades and petioles of the carrot (*Daucus carota*), and *Daucus maritimus, D. pulcherrimus, D. pusillus, D. hispanicus* and *D. gingidium.* No marked resistance to Cercospora blight was found among 112 carrot varieties, selections, and *Daucus* species tested in the field.

The germ tubes enter the host through the stomata. The advancing hyphae are intercellular at first, but intracellular invasion soon occurs. Sporulation may occur soon after the fungus establishes itself in the substomatal cavity, but more often after the mycelium has invaded more deeply into the host. The fungus invades all of the epidermal and parenchymatous tissue between the two surfaces of the leaf blades, but in the petiole seldom penetrates very deeply until the leaf begins to die.

Most rapid growth of the fungus in culture occurred between 19° and 28° C. Maximum germination of conidia occurred between 16° and 28° C., and abundant conidia production between 13° and 28° C. Maximum growth in culture was made between pH 5.5 and 7.0.

The fungus may persist in the soil from one crop to another and may be spread to other fields by wind.

Infected seed umbels have been observed and viable conidia are found on the seed from such plants. Carrot seed, artificially contaminated with conidia of the fungus, treated with ethyl mercury phosphate and ethyl mercury tartrate either as a liquid (1–24,000) or a dust, mercuric chloride (1–1000) and Spergon remained free of fungous growth when placed on sterile potato-dextrose agar. The effect of several seed treatments on percentage stand has been determined in the greenhouse.

Number of leaf spots per cm.$^2$ did not differ significantly on carrots complete in nitrogen, phosphorus, and potassium, deficient in potassium, or

deficient in phosphorus. The calcium-deficient and the nitrogen-deficient plants had significantly fewer leaf spots per cm.$^2$ when compared with the complete-nutrient plants. The leaf spots attained their greatest length on the complete-nutrient plants, were intermediate in length on the potassium-deficient and the phosphorus-deficient plants, and were shortest on the calcium-deficient and the nitrogen-deficient plants. Conidia were most abundant on the lesions on the complete-nutrient plants, less abundant on the potassium-deficient plants, and least abundant on the phosphorus-deficient, the calcium-deficient, and the nitrogen-deficient plants.

When the fungus was grown on the sterile leaf-juice extracts obtained from plants deficient in potassium, phosphorus, calcium, or nitrogen and plants complete in these elements, more growth was obtained on the leaf extracts from the complete, the potassium-deficient, and the nitrogen-deficient plants than on extracts of the calcium-deficient and the phosphorus-deficient plants.

## LITERATURE CITED


1. DORAN, W. H., and E. F. GUBA. Blight and leaf spot of carrots in Massachusetts. Mass. Agr. Exp. Stat. Bull. 245: 271–278. 1928.
2. GODFREY, G. H. Notes on vegetable diseases in the lower Rio Grande valley in the spring of 1938. Pl. Dis. Rptr. 22: 122–124. 1938.
3. HOAGLAND, D. R., and D. I. ARNON. The water-culture method for growing plants without soil. Univ. of Calif. Agr. Exp. Stat. Cir. 347, 49 pp. 1938.
4. KLOTZ, L. J. A study of the early-blight fungus, *Cercospora apii*. Mich. Agr. Exp. Stat. Tech. Bull. 63, 44 pp. 1923.
5. LEHMAN, S. G. Frog-eye (*Cercospora diazu* Miura) on stems, pods, and seed of soybean, and the relation of these infections to the recurrence of the disease. Jour. Agr. Res. [U. S.] 48: 131–147. 1934.
6. MCKAY, M. R., and V. W. POOL. Field studies of *Cercospora beticola*. Phytopath. 8: 119–136. 1918.
7. NAGEL, C. M. The longevity of *Cercospora beticola* in soil. Phytopath. 28: 342–350. 1938.
8. POOL, VENUS W., and M. B. MCKAY. Climatic conditions as related to *Cercospora beticola*. Jour. Agr. Res. [U. S.] 6: 21–60. 1916.
9. ———. Relation of stomatal movement to infection by *Cercospora beticola*. Jour. Agr. Res. [U. S.] 5: 1011–1038. 1916.
10. PRYOR, DEAN E. The effect of some mineral nutrients on the development of club-root of crucifers. Jour. Agr. Res. [U. S.] 61: 149–160. 1940.
11. ROLDAN, E. F., and A. F. QUERIJERO. Black spot of peanut. Philippine Agricult. 27: 669–682. 1939.
12. STEWART, F. C. Notes on New York plant diseases. N. Y. (Geneva) Agr. Exp. Stat. Bull. 328: 305–404. 1910.
13. THORNTON, S. F., S. D. CONNER, and R. R. FRASER. The use of rapid chemical tests on soils and plants as aids in determining fertilizer needs. Ind. Agr. Exp. Stat. Cir. 204, 16 pp. 1936.
14. WOLF, F. A. Further studies on peanut leaf spot. Jour. Agr. Res. [U. S.] 5: 891–902. 1916.
15. WOODWARD, R. C. *Cercospora fabae* Fautrey, on field beans. Trans. Brit. Myc. Soc. 17: 195–202. 1932.

# THE PRESENCE OF A TOXIN IN TOMATO WILT[1]

DAVID GOTTLIEB

(Accepted for publication June 12, 1942)

## INTRODUCTION

Although the fungi that cause many vascular wilts are well known, the mechanism by which wilting occurs remains a moot problem. Many theories have been formulated to account for loss of turgidity by the plants; the earliest of these hypothesized the plugging of vessels. Massee (14), working on the sleepy disease of tomatoes, and, shortly afterwards, Smith (17), investigating the wilts of cotton, watermelon, and cowpea, observed that many of the tracheae in the hosts were filled with hyphae. From this evidence they postulated the theory that water was unable to pass through the xylem in sufficient quantities to keep the leaves turgid. Therefore, the plants died of drought. Brandes (4), and Ahmet (1), however, point out that mycelium usually is not present in sufficient quantities to hinder the passage of water in the tracheae. Clayton (5) tested the plugging hypothesis by girdling portions of tomato stems, but was unable to obtain a unilateral wilt, such as has been observed often in the field. Because of the resemblance borne by tomato wilt to the symptoms obtained when poisons are injected into plants, Clayton believed that toxins produced by the pathogen caused the wilting. Evidence was slowly accumulated that many of the wilt organisms produce poisons in synthetic media (2, 5, 6). When stems of healthy plants were placed in the filtrates of nutrient solutions in which the pathogen had been grown, they wilted; and the vascular tissues were discolored. From such data the theory that a toxin produced by the fungus causes vascular wilts has gained credence. In this paper the term toxin is not used in its strict medical meaning, but will indicate a poisonous substance produced in the plant due to the presence of the fungus. Good reviews of literature on the subject of fungus toxins are given by White (20), Grossman (9), and Fisher (8). Many investigations have been made concerning the nature of the toxin found in the synthetic media, but the active principle has not yet been identified (7, 13, 16). Still another explanation of the cause of wilting has been advanced by Tochinai (18). In a study of the physiology of *Fusarium lini* he discovered that a large amount of carbon dioxide was produced by the parasite. This, he claimed, could form gas pockets in the vessels, which might stop the movement of water through the stem, thereby causing the wilting of flax.

Evidence for the existence of fungal toxins has been obtained by growing the fungi in synthetic media. Such evidence is not entirely conclusive. It is well known that materials produced by fungi will vary with the substrate

[1] Paper No. 2003, Scientific Journal Series, Minnesota Experiment Station. Assistance in the preparation of figures 2, 3, 5 was furnished by the personnel of the Works Projects Administration, Official Project No. 165–71–1–124, Sponsored by the University of Minnesota (1942).

on which they are grown (3). Rosen (15), for example, found that *Fusarium vasinfectum* produces a toxic substance when grown in Richards' solution, but not when grown in nutrient broth or Uchansky's solution. Since the environment and nutrients in the tracheae of a plant are very different from those of a synthetic nutrient solution, there is a possibility that no toxic materials or that different toxins are produced by the fungus in the host plant. Hursh (12) sums up the present status of the problem very aptly:

"It was postulated that the pathologic condition in plants infected with certain wilt disease might be brought about by some fungus products deleterious to the host tissue that had been transported considerable distances through the stem from the point of attack. Should the presence of such

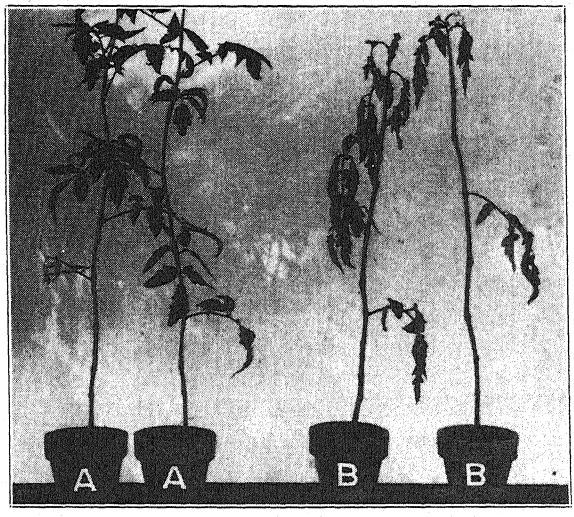



FIG. 1. Tomato plants used for the extraction of tracheal fluid. A. Noninoculated turgid plants. B. Inoculated wilted plants.

substances actually be demonstrated it would be an important contribution to an understanding of the physiology of fungus parasitism." With this in mind, the object of the present research was to ascertain the presence or absence of toxins as a factor in the fusarial wilt of tomatoes.

## MATERIALS AND PROCEDURE OF THE PRESENT WORK

The Bonny Best variety of tomato was used as the host and a virulent isolate of *Fusarium bulbigenum* var. *lycopersici*[2] as the pathogen. Tomato seeds were planted in flats containing a steam-sterilized mixture of 2 parts silt loam and 1 part sand. After the first true leaves appeared, the seedlings were transplanted to 4-inch pots and grown until approximately 45 cm. tall. The plants were then inoculated with a culture of the *Fusarium* which had been grown in Richards' solution for two weeks. Wellman's (19) method

[2] The isolate of *Fusarium bulbigenum* var. *lycopersici* was obtained through the courtesy of Dr. F. L. Wellman, U. S. Department of Agriculture.

of inoculation was used, and the plants were immediately repotted. They were covered with manila paper for 48 hours before exposure to direct sunlight. Unless this precaution was taken the tomatoes received a severe shock, resulting in injury that might have been confused with the disease. The temperature in the greenhouse varied from 75 to 87° C., and moisture in the soil was kept just under the saturation point. Check plants were treated similarly except that their roots were placed in sterile water instead of in the inoculum. Within 21 days after inoculation most of the inoculated plants had wilted, whereas the checks remained turgid (Fig. 1). The vascular systems of all the diseased plants were discolored; those of the healthy plants were not.

### THE EXTRACTION OF PLANT-CELL SAPS AND WILTING OF TOMATO SEEDLINGS IN THE EXTRACTS

The tomato plants were placed in a freezing chamber for 24 hours. The leaves were then stripped off and the stems cut into small sections. A hydraulic press was used to express the sap under pressure ranging from 250 lb. to 15,000 lb. per sq. in. The sap was next centrifuged at 2500 r.p.m. for 30 minutes and the supernatant fluid freed of the pathogen by passage through a Berkefeld filter. The filtered sap was transferred to small tubes 5 mm. in diameter into which the shoots of tomato seedlings 10 cm. tall were placed and observed for wilting.

No consistent differences in time required for wilting could be observed between the seedlings in the sap of diseased plants and those in the sap of healthy plants. Both saps caused wilting within 4 hours. An absolute check of sterile water also was run. No wilting, however, was observed in the checks even after 24 hours. To determine whether the wilting in the sap might be due to an effect of osmotic pressure, the sap was diluted as much as 1 to 8 with distilled water. Even in such low concentrations, however, the plants wilted within 4 hours. This indicated that some factor other than a high osmotic value was responsible for the toxic condition of the saps of both normal and diseased plants when they were thus extracted. Sap obtained at a pressure of 250 lb. per sq. in. proved less toxic than that obtained at a pressure of 15,000 lb.

It was apparent that if any toxin with a capacity for wilting tomato seedlings had been secreted by the parasite in the diseased plant, its action was masked by other injurious substances formed or secured during the extraction of plant saps. Such poisons might be formed by the release of some cellular contents and their chemical change upon death or injury to the plants. In his investigations on wilt of potatoes, Haskell (11) has evidence to support this possibility. He discovered that when a portion of the stem was killed with steam some poisonous materials were formed that discolored the vascular system, as occurs in the fusarial wilts. To preclude the effect of such poisonous agents in the sap, a method was devised for using tracheal fluids rather than the expressed sap.

CENTRIFUGING TRACHEAL FLUIDS

Since the pathogen is a vascular parasite, it seems reasonable to assume that any toxins present should first appear in the vascular fluids. An assay of these fluids should reveal the presence or absence of the toxin involved in tomato wilt. Centrifuging the vascular fluids from the stems of the plants was decided on as offering the most feasible means of obtaining these liquids.

## Apparatus

Centrifuge tubes similar to those described by Hamm *et al.* (10) for spectrographic analysis were used. These had been previously modified so

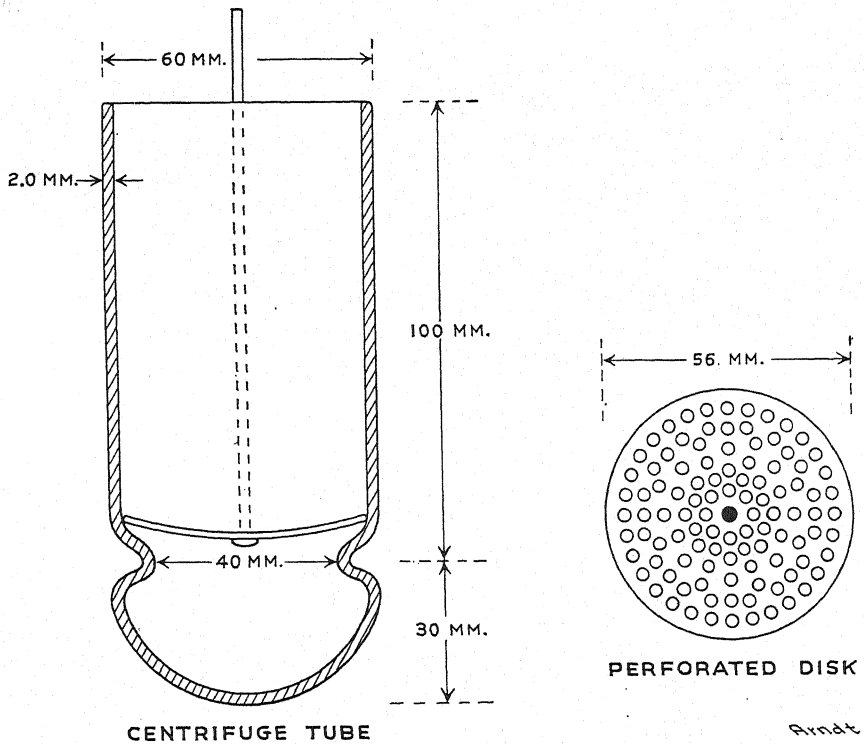



FIG. 2. Centrifuge tube.

that the stems were contained in a glass inner tube with a small hole at the closed end through which the liquid escaped into the collecting chamber. Because of the extremely small amounts of fluid obtained by centrifuging tomato stems, a different type and much larger tube was designed[3]. The vessel was constructed of a section of heavy walled pyrex tube 130 mm. long and 60 mm. in diameter. One end of the tube was sealed to form a rounded bottom, and a collar was blown into the inside of the vessel, 30 mm. from the bottom. This shelf supported a removable stainless-steel plate 55 mm. in diameter and into which 100 2-mm. holes had been drilled. A steel rod was riveted to the center of the plate to facilitate removing the stems from the

[3] The aid of Dr. Richard Nelson in designing this tube is greatly appreciated.

centrifuge tube. The diaphragm was covered with glass wool to filter off any solid particles dislodged from the stems during centrifuging (Fig. 2).

Since chemical changes might occur during the extraction, the entire experiment was run under an atmosphere of nitrogen. The joints of the centrifuge were sealed with glazier's putty and the cover sealed with petrolatum. A water trap was placed between the nitrogen tank and the centrifuge to aid in regulating the flow of gas. The outlet of the trap was provided with a 3-way stopcock; one of the arms led to the centrifuge chamber and the other to a pipette used for flushing the centrifuge tubes free of air before each run. This same outlet was used when filtering the tracheal fluids anaerobically. The entire apparatus is illustrated in figure 3. An

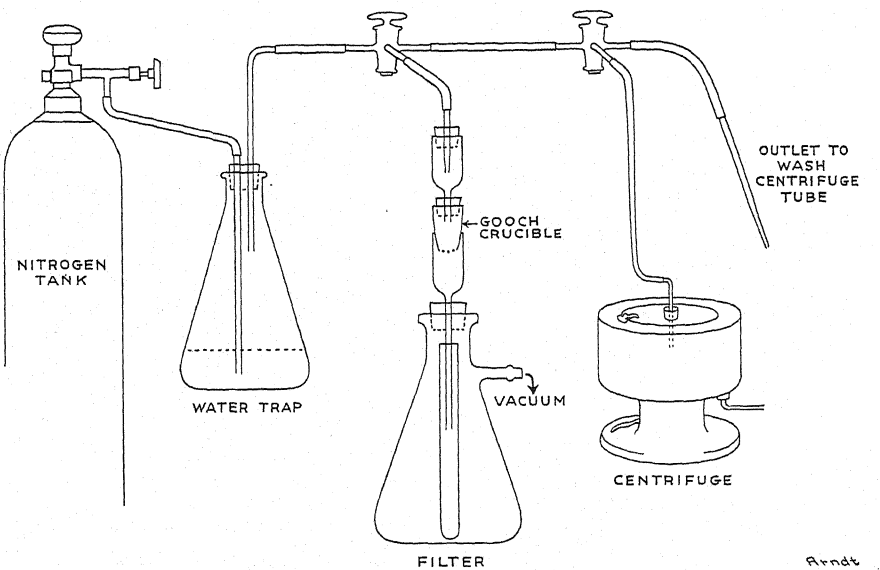



FIG. 3. Apparatus for centrifuging and filtering tracheal fluids.

analysis of the gaseous contents of the centrifuge after some sample runs, showed the presence of only 2.01 per cent of 2.12 per cent oxygen, with an average 2.03 per cent.

## Procedure

The plants were grown and inoculated as described in the previous experiment. After they had wilted, the leaves were removed and the stems cut into pieces 8 cm. long. These were packed in the tubes, which were then flushed with nitrogen and centrifuged. The centrifuge was washed with nitrogen for 40 minutes prior to addition of the samples and the gas continued to flow into the machine during the entire extraction. After centrifuging for 20 minutes, the liquid was removed from the collecting tubes and stored under nitrogen at $-18°$ C. for 12 to 24 hours. The tracheal fluids later were filtered anaerobically.

Small vials made of 4-mm. glass tubing, 50 mm. tall, having a capacity

of 1.5 ml., were used to hold the tracheal fluids and the seedlings during the tests. Large corks were found to be excellent bases for them. One ml. of the filtrate was placed in each test vial. Seedlings 3 weeks old and 6 to 8 cm. tall, whose stems had been cut under water, were used to test for the presence of a toxin, one seedling being placed in each vial. These assays were run in the laboratory under an illumination of 405 foot candles obtained from 2 100-w. Mazda lamps at a height of 60 cm. from the table. The temperature varied between 26 and 28° C. The time required for wilting of seedlings in the vials was observed. Tracheal fluids from the uninfected tomatoes were used as checks. To determine whether the tracheal fluid of normal plants affected the seedlings, absolute checks were run with distilled water.

### Precautions Taken in Selecting Plant Materials

The plants were examined for vascular discoloration before using them in this work. All 152 of the inoculated plants had brown vascular bundles

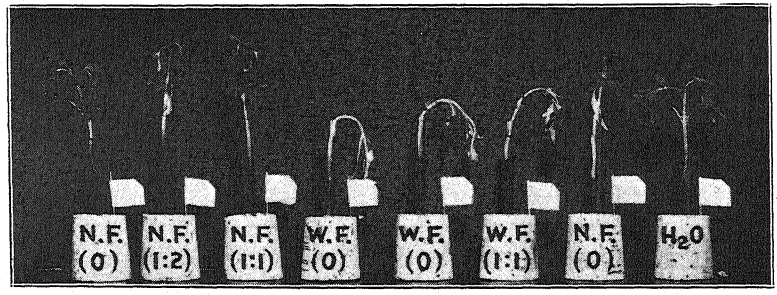



FIG. 4. Assay for toxin. Vials labeled N.F. contain tracheal fluid from noninoculated turgid plants. Vials labeled W.F. contain fluids from plants which were inoculated with *Fusarium bulbigenum* var. *lycopersici* and which had wilted.

at time of wilting. This discoloration was absent in the 103 noninoculated plants. Microscopic examination of free-hand sections from stem and root tissues fixed in Formalin, alcohol, acetic acid solution, showed that the tracheae of the inoculated plants contained mycelium; whereas, no mycelium was found in the noninoculated ones. Isolations on potato-dextrose agar were made from the same plants and a *Fusarium* was obtained from all the material that had been inoculated. No *Fusarium* was isolated from tissues of healthy plants. Mycelium from the isolations was reinoculated into healthy plants and caused a typical wilt.

### THE EFFECTS OF TRACHEAL FLUIDS ON TOMATO SEEDLINGS

A total of 42 seedlings, divided into 3-series, was used to determine the toxicity of the vascular fluids. Sixteen seedlings were placed in the extract of the diseased plants, 14 in the extracts of normal plants, and 12 in distilled water. In each series the seedlings in the liquid obtained from the diseased plants wilted long before those in the fluid from the healthy plants, the time required varying from series to series, from 1 hour to 7 hours (Fig. 4); but

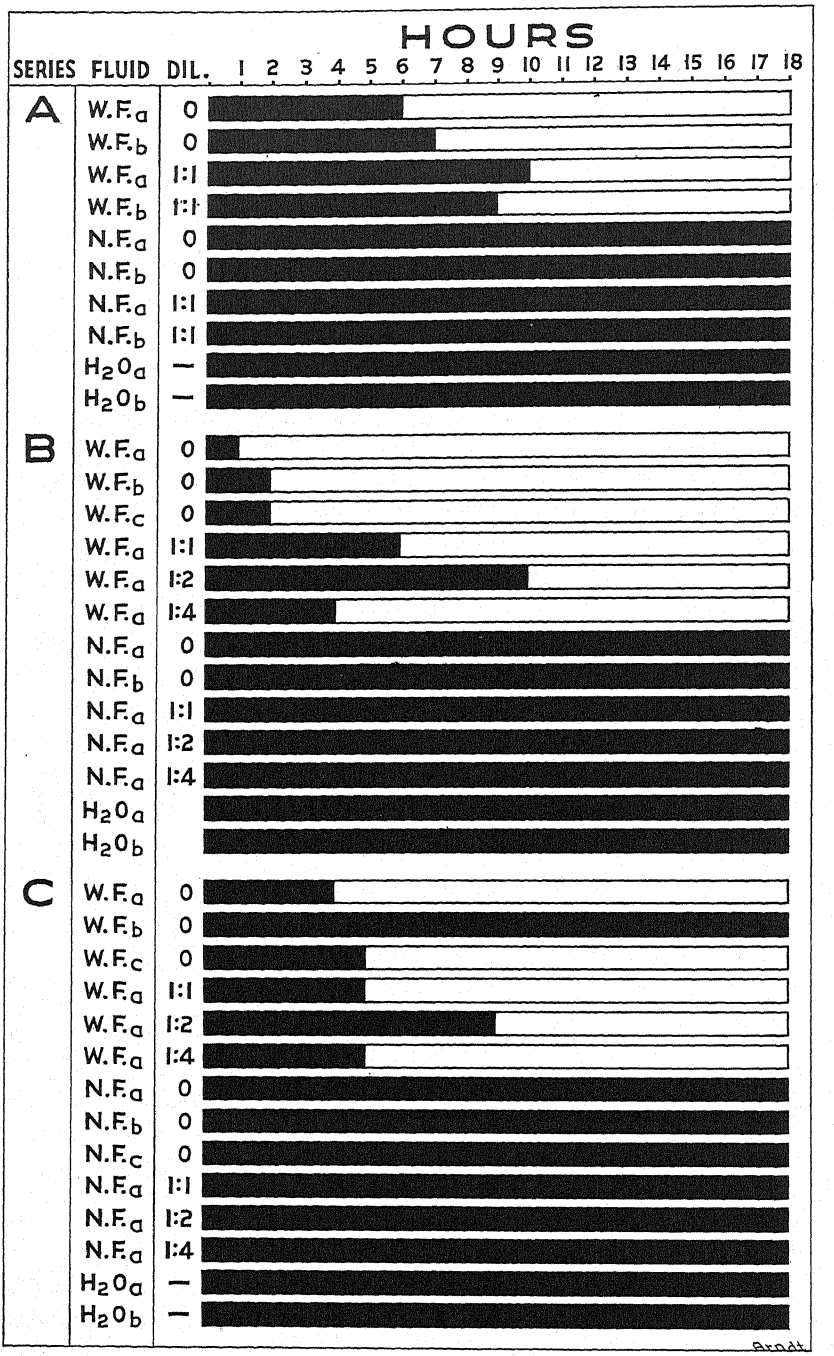



FIG. 5. Chart showing time required for tomato seedlings to wilt. Black bar denotes turgidity; white bar indicates a wilted condition. Fluids N.F. were obtained from non-inoculated, turgid plants. Fluids W.F. were obtained from inoculated wilted plants.

in each series, the replicates lost their turgidity within 1 hour of each other. Only 1 seedling in 1 series, W.F.$_b$ (0 dil.) in series C, failed to wilt; it was only slightly flaccid even after 19 hours. All the seedlings in the fluids of normal plants and those in distilled water remained turgid during the entire course of the analysis (Fig. 5). Dilution of the fluids from diseased plants affects wilting. Although seedlings wilted much sooner in a zero dilution of the extract than in greater dilutions, there was no significant correlation between the rate of dilution and the rapidity of wilting. The results indicate that there is some toxic material in the tracheal fluids of tomato plants infected with *Fusarium bulbigenum* var. *lycopersici.*

### TRACHEAL FLUIDS FROM PHYSIOLOGICALLY WILTED PLANTS

Because of the chance that the toxic character of the tracheal fluids was due to a generalized physiologic reaction of the host to the process of wilting, and not specifically to the presence of the parasite, another series of experiments was planned. In this series the vascular fluids of normal plants were compared with those of tomatoes which had been deprived of water so that they were in an advanced stage of wilting. Eight seedlings were placed in each of the different fluids. All the seedlings in the extract from the normal, turgid plants and all in the extract from tomatoes which had wilted because of insufficient water, remained turgid even after 24 hours. Thus, a physiological wilting in itself does not result in the presence, in tracheal fluids, of materials toxic to young tomato seedlings. We may justly conclude that the toxin present in tracheal fluids from plants wilted by *Fusarium bulbigenum* var. *lycopersici* must be due to the activity of the fungus.

### DISCUSSION

From the results obtained in this investigation, it is evident that a toxin is present in the tracheal fluids of tomatoes showing fusarium wilt. The active constituent, moreover, is associated definitely with the presence of the fungus, *Fusarium bulbigenum* var. *lycopersici*, for, when wilting was induced in absence of the pathogen no toxin could be found in the fluids. This evidence is in line with past researches showing that the fungus produces poisonous substances in artificial media. However, the identity of the toxins in the tracheal fluid and those in the synthetic media has not been ascertained. Rosen (15) has criticized the evidence obtained by growing the pathogen in nutrient solutions. He found a toxin when *Fusarium vasinfectum* was grown in some synthetic media but none when grown in others. He, therefore, raised the following questions as to criteria for valid evidence: "It opens up the whole question as to the significance to be attached to finding of toxic properties with any medium which does not closely approach the chemical and physical makeup of the natural host."

The techniques used in the present study for demonstrating the role of toxins in tomato wilt ought to fulfill, to some extent, these criteria. Since the fungus inhabits the lumen of the xylem, the xylem fluid is the natural medium for the growth of the parasite and the production of toxin. In

addition, the method of extraction precluded, so far as possible, the opportunity for chemical changes of the fluids.

Still, there is a chance that the toxic material is not a direct product of the fungus metabolism. This component of the vascular fluid might result from host cells killed or injured and disorganized by the penetration and progress of the fungus. Haskell's (11) evidence for the production of poisonous substance by steam-injured stems indicates this possibility must not be overlooked. However, separation of the direct and indirect effects of the fungus would be exceedingly difficult to demonstrate.

The centrifuging technique should prove valuable in the study of vascular parasites, such as are involved in Dutch Elm disease, Verticillium wilt of maple, potato wilt, and many others in which toxins are suspected. By this means the tracheal fluids can be obtained with a minimum of mixture with cell sap and with relatively little chemical change. Among its advantages over the sap-displacement procedure are the short time required for the extraction, the fact that there need be no dilution with water, and that the entire process can be run under anaerobic conditions. In addition, the procedure can be adapted for herbaceous as well as woody plants. The only requirements are that the stem be sufficiently sturdy and that there be a sufficient number of vessels with a bore large enough so that the forces of capillarity can be overcome by centrifuging. Therefore, it could not be used successfully on such crops as wheat and flax.

## SUMMARY

A method has been devised for collecting anaerobically the tracheal fluids of plants.

Tomato plants wilted by *Fusarium bulbigenum* var. *lycopersici* contain a toxin in their vascular fluids.

No toxins were found in tomato plants wilted by lack of sufficient soil water.

When the cell sap of either healthy tomato plants or those with Fusarium wilt is expressed from the stems under aerobic conditions, a poisonous substance is present that causes tomato seedlings to wilt when placed in the sap.

The writer wishes to express his thanks to Dr. E. C. Stakman and Dr. Helen Hart for their many helpful suggestions; to Dr. Ian W. Tervet for his interest and encouragement during the course of the research; and to Betty Gottlieb for her aid in carrying out this investigation.

## LITERATURE CITED

1. AHMET, HIKMET. Untersuchungen über Tracheomykosen. Phytopath. Zeitschr. 6: 49–101. 1933.
2. BEWLY, W. F. Sleepy disease of tomatoes. Ann. Appl. Biol. 9: 116–134. 1922.
3. BLANK, L. M., and P. J. TALLEY. The carbon utilization and carbohydrase activity of *Phymototrichum omnivorum*. Amer. Jour. Bot. 28: 564–569. 1941.
4. BRANDES, L. W. Banana wilt. Phytopath. 9: 339–389. 1919.

5. CLAYTON, E. E. The relation of temperature to Fusarium wilt of tomato. Amer. Jour. Bot. 10: 71–88. 1923.
6. COONS, G. H. Report of the Botanist. Mich. State Bd. Agr. Ann. Rpt. 55: 265–271. 1916.
7. FAHMY, T. The production by *Fusarium solani* of a toxic excretory substance capable of causing wilting in plants. Phytopath. 18: 543–550. 1923.
8. FISHER, P. L. Physiological studies on the pathogenicity of *Fusarium lycopersici* for the tomato plant. Md. Agr. Exp. Stat. Bull. 374. 1935.
9. GROSSMAN, H. Untersuchung über die Welkenkrankheit des Flachses. Phytopath. Zeitschr. 7: 545–583. 1934.
10. HAMM, P., R. B. HARVEY, and E. S. MILLER. Quantitative spectroscopic analysis of stem "tracheal" fluids for inorganic constituents. Proc. Soc. for Experimental Biol. and Medicine 46: 347–351. 1941.
11. HASKELL, R. J. Fusarium wilt of potatoes in the Hudson River Valley, New York. Phytopath. 9: 223–260. 1919.
12. HURSH, C. R. The reactions of plant stems to fungus products. Phytopath. 18: 603–610. 1928.
13. LATHROP, E. C. The generation of aldehydes by *Fusarium cubense*. Phytopath. 7: 14–16. 1917.
14. MASSEE, G. A disease of tomatoes. Gard. Chron. Ser. 3 17: 707–708. 1895.
15. ROSEN, H. G. Efforts to determine the means by which the cotton wilt fungus, *Fusarium vasinfectum*, induced wilting. Jour. Agr. Res. [U.S.] 33: 1143–1162. 1926.
16. SCHAFFNIT, E., and M. L. LUDTKE. Ueber die Bildung von Toxinen durch verschiedene Pflanzenparasiten. Ber. Deutsch. Bot. Ges. 50: 444–463. 1932.
17. SMITH, E. F. Wilt disease of cotton, watermelon, and cowpea (*Necomospora* nov. gen.). U. S. Dept. Agr. Div. Veg. Path. Bull. 17. 1899.
18. TOCHINAI, YOSHIHIKO. Comparative studies of the physiology of *Fusarium lini* and *Colletotrichum lini*. Jour. Coll. Agr. Hokkaido Imperial Univ. 14: 171–236. 1925. (Rev. Biol. Abst. 1: 25. 1926–27. (Original not seen.)).
19. WELLMAN, F. L. A technique for studying host resistance and pathogenicity in tomato Fusarium wilt. Phytopath. 29: 945–956. 1939.
20. WHITE, R. P. Studies on tomato wilt caused by *Fusarium lycopersici* Sacc. Jour. Agr. Res. [U.S.] 34: 197–239. 1927.

# STAMEN BLIGHT OF BLACKBERRIES[1]

S. M. Zeller and Alvin J. Braun

(Accepted for publication May 28, 1942)

Stamen blight of blackberries caused by *Hapalosphaeria deformans* Sydow was first observed in Oregon in 1937 on Young dewberry grown at Kellogg, Douglas County (5). The wild Trailing blackberry (*Rubus macropetalus* Doug.) growing along nearby fences also was infected. The following year infected Young and Boysen dewberries were discovered by F. D. Bailey at North Albany. A year later scattered infections were observed on several commercial plantings of Evergreen blackberry (*R. laciniatus* Willd.), in Marion County. The disease is widely distributed throughout the Willamette Valley on Young and Boysen dewberries, and the Evergreen blackberry.

Stamen blight of Loganberries was reported in British Columbia by Dearness and Foster in 1933 (3). In a recent communication from Glenn A. Huber of the Western Washington Experiment Station (Puyallup) record is given of *Hapalosphaeria deformans* on the Young (Olympic) variety of blackberry in the Puget Sound area. It is evident, therefore, that this disease is widely distributed throughout the Pacific Northwest, especially west of the Cascade Mountains.

In Europe the first published record of the stamen-blight organism was by H. and P. Sydow (1) under the new name *Paepalopsis deformans* (1907). The type locality was Thuringia, Germany, on *Rubus caesius* L. (syn. *R. dumetorum* Weihe). There is also a report (4) of this disease on *R. fruticosis* L. in England in 1904. Diedicke and Sydow (2) first illustrated the fungus within anthers.

## SEVERITY OF THE DISEASE

The most abundant infection we have observed involved about 70 per cent of Young dewberry blossoms in a 6-acre commercial planting in 1937. The long life of such plantings and their concentration in culture ordinarily should favor cumulative epidemics in succeeding seasons. Seasonal epidemics of this pathogen, however, are greatly influenced by the fluctuation of aerial environments. Thus in 1938 the above-mentioned planting showed but 15 per cent infection and up to about 30 per cent one season since. Consequently, when conditions are favorable stamen blight has potentialities for serious berry-crop losses.

## DESCRIPTION OF THE DISEASE

In the spring, when the flowers of the host open, the fungus is most readily detected. When the petals first unfold the diseased anthers already have a moldy appearance due to white masses of spore coils (Fig. 1). Soon after

[1] Published as Technical Paper No. 412, with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany.

FIG. 1. Young dewberry flowers in two stages of blooming. The two above infected with *Hapalosphaeria deformans*. Note the snowy-white coils of spores exuding from pycnidia in the anthers. These cirri are especially prominent immediately after the flowers open. Note also that the infected anthers do not dehisce, as do the healthy (lower right).

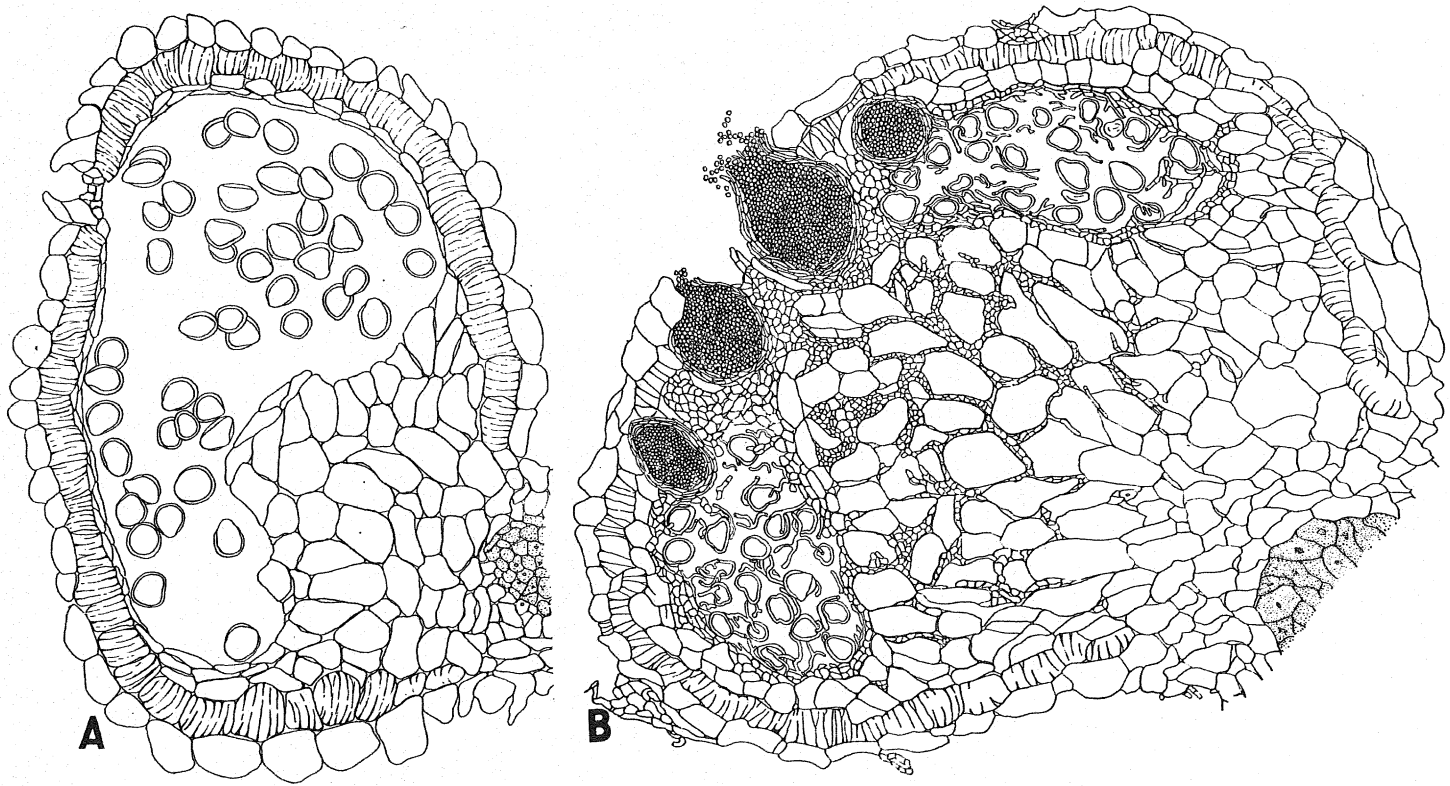



FIG. 2. A. Cross section through a healthy Young dewberry anther. B. Cross section through a Young dewberry anther infected with *Hapalosphaeria deformans*, showing the mycelium in the tissue and between the pollen grains, as well as the stromatic pseudoparenchyma lining the pollen chamber and upon which the spore-filled pycnidia are seated. (Both drawings by Mrs. D. P. Rogers; both $\times 120$.)

the healthy flowers open, their anthers dehisce, exposing cream-colored pollen. In contrast, diseased anthers never dehisce but are covered with white spore horns of the fungus.

The diseased blossoms, completely emasculated by the fungus, usually do not develop normal fruits, since blackberry flowers generally are self-pollinated. Some of these bee-pollinated flowers may produce deformed berries. These are usually unsuitable for canning (Fig. 3).

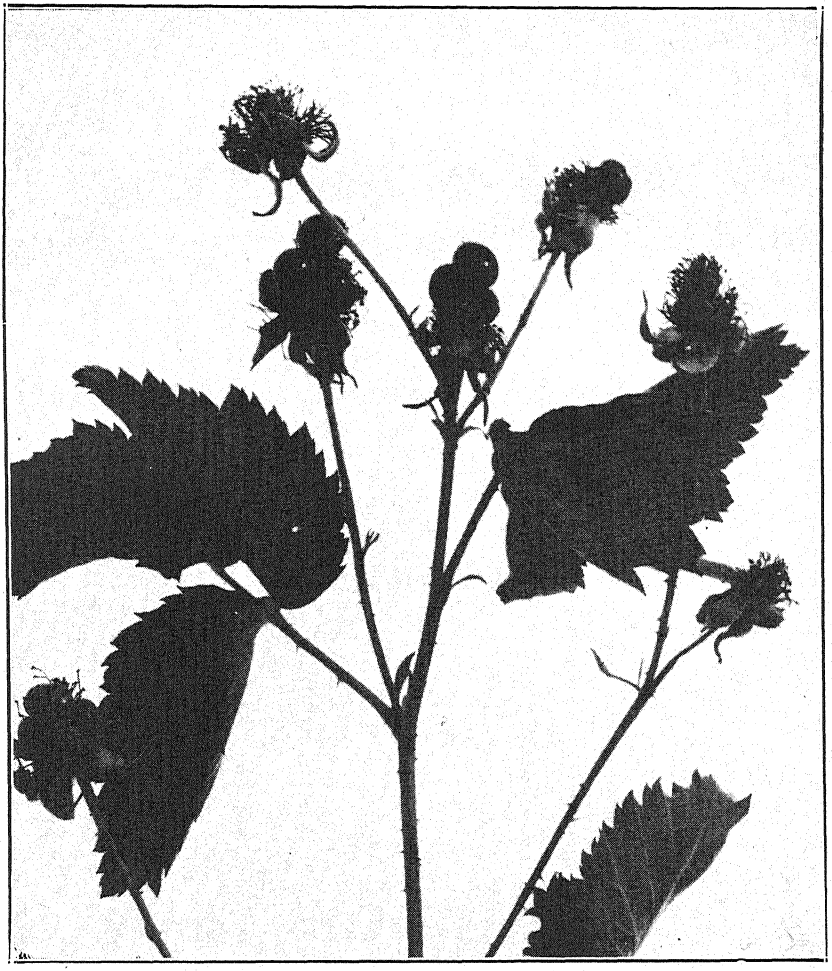


FIG. 3. Poorly developed berries from blossoms of Young dewberry infected with *Hapalosphaeria deformans*.

Diedicke and Sydow (2) report that *Hapalosphaeria deformans* causes a type of witches' broom in the fruiting laterals of *Rubus caesius*. This proved true also of infected laterals of *R. macropetalus* in Oregon. There is no evidence, however, of such anomalous growth of infected axillary laterals of fruiting canes of Boysen dewberry, Evergreen blackberry or Young dewberry.

The fact that the flowers on a fruiting lateral usually are all infected indicates infection of the unfolded axillary bud. An individual fruiting cane, however, may bear both healthy and diseased laterals.

## THE CAUSAL FUNGUS

In 1907, H. and P. Sydow (1) first described the hyphomycetous form of the causal fungus as *Paepalopsis deformans,* on *Rubus caesius* L. The following year Diedicke (2) discovered pycnidia in the anther walls, and Sydow (2) created the new monotypic form genus, *Hapalosphaeria,* in the Sphaeropsidales to accommodate *P. deformans.*

The mycelium in the anther tissues is hyaline, septate, 2–4 μ diam., irregularly branched, and confined to the intercellular spaces. Strands of mycelium form a loose network among the pollen grains within the locules of the anthers. The fungus forms a pseudoparenchyma about 2–5 cells thick completely lining the walls of the pollen sacs (Fig. 2, B). This not only prevents the dehiscence of the anthers and escape of the pollen but also the complete infection of the anther walls starves the developing pollen. Pycnidia with brownish walls are formed in the anthers seated on the pseudoparenchymatous layer and opening to the outside of the anthers. Upwards of 50 pycnidia may be found in one anther.

The subglobose or ovoid pycnidia, 50–110 μ in diameter, are filled with subglobose, hyaline pycnidiospores which are about 2.5–4.9 μ in diameter. These spores are discharged in white coils, which give the anthers the white, moldy appearance (Fig. 1) mentioned above.

The fungus may be readily cultured on potato-dextrose agar. Anthers dissected aseptically from closed buds parasitized by the fungus have usually yielded pure culture of *Hapalosphaeria deformans* when planted on the above medium. Pycnospores from such flowers when planted in agar Petri plates also give pure cultures.

Pure cultures grow slowly on agar, and thus the colonies form firm, compact, dark-gray mats. The agar directly beneath these mats and around their edges becomes discolored with shades from yellow to deep-Burgundy, each isolate imparting to the agar its own particular hue throughout a series of subsequent transfers. At times a whiter strain may be isolated but, so far as known, these light-colored strains may not differ in other respects from the usual dark-gray strain.

No spores or sclerotia have been formed in the pure cultures of the fungus, even during periods as long as 1 year.

Infected Young dewberry anthers were overwintered in cloth bags on and above the ground in an attempt to discover a perfect stage of *Hapalosphaeria deformans.* No such stage was found. After 3 years of unsuccessful attempts it is concluded that if there is a spore form other than the pycnidial stage it is formed very rarely and under unusual conditions. At any rate, it can be assumed that the perfect stage is not essential to the annual epidemiology of the disease, especially as indicated by field experiments

reported later in this paper. Anthers that were not badly contaminated with mold after exposure to field conditions during the winter, readily yielded cultures of *H. deformans* when plates were poured from agar suspensions made by crushing the anthers. This indicates a rather long life of the thin-walled pycnidiospores, at least when protected within the pycnidia.

### EXPERIMENTS ON FIELD INFECTION

After unsuccessful attempts to obtain a perfect stage of the causal organism during two winters, it seemed probable that the pycnidiospores produced in the anthers at blossoming time must be capable of infecting the axillary buds of the current-season canes. Two experiments were set up at Kellogg, Oregon. In one experiment axillary buds of Young dewberry were dusted with spores from infected blossoms at the peak of the 1940 flowering season, when the new cane growth was about 3 to 4 feet long.

Approximately 75 per cent of the blossoms had opened by May 8. At this time 5 buds per cane on each of 40 canes were inoculated by lightly tapping an infected blossom against an axillary bud just becoming evident in the axil of the leaf. A good spore load for each bud was obtained by using 1 infected blossom for each set of 2 or 3 buds. Each of the 40 canes was tagged for identification. On May 9, 1941, a count of the number of infected laterals growing from the dusted axillary buds was made. The results of this experiment are given in table 1.

TABLE 1.—*Data taken May 9, 1941, on flowering laterals of Young dewberry originating from axillary buds dusted with pycnidiospores of Hapalosphaeria deformans the preceding year (May 8, 1940)*

| Plant structures dusted | Total counted | Infected | Percentage of infection |
|---|---|---|---|
| (1) Flowering laterals from axillary buds dusted with spores in 1940 | 127 | 53 | 41.7 |
| (2) Flowering laterals from axillary buds not dusted with spores in 1940. [Same plants as in (1)] | 1000 | 55 | 5.5 |
| (3) Natural infection of flowering laterals in plants near those dusted with spores | 1000 | 55 | 5.5 |

In a second experiment all of the canes, young as well as fruiting, were removed from a row of 24 Young dewberry plants in a portion of the field showing 21 per cent infection. The canes were cut out on August 2, 1940. Since the new canes, produced late in the season, attained lengths of not more than 2 to 6 feet, they supported relatively few axillary buds. Five hundred flowering laterals from these canes, observed on May 9, 1941, revealed *no* infection, whereas plants normally treated on either side showed from 6 to 8 per cent infection.

The data presented in table 1, as well as the results of the pruning ex-

periment, indicate rather conclusively that axillary buds of current-season canes are infected directly by pycnidiospores or perchance by sporidia produced from them. It may also be observed from these experiments that the source of inoculum of this disease probably is limited to the pycnidiospore or its derivatives. The period of inoculum production is essentially that of and just following the blossoming period of the host plant. Since, as stated above, pycnidiospores may withstand the drouth of summer and the rigors of winter, it is not yet known just when bud infection by *Hapalosphaeria deformans* actually takes place between May and the following March. It seems probable that the pycnidiospores lie dormant within the leafy scales of the winter buds and do not germinate until late in February or early March.

Since the blackberry does not possess a tightly closed scaly bud, infection undoubtedly occurs through the more or less open apex. The first evidence of bud infection, as observed through a study of histological sections, was seen in March. A slight, scattered network of mycelium between the flower buds within the axillary winter bud was then discovered. Then, within the flower buds, a very sparse growth of mycelium may be found among the flower parts. Histological sections, however, have revealed no floral parts infected, except the anthers; nevertheless hyphae may be found growing over and between the pistils in later stages. Since there is no infection of the stamen filaments, incipient infection of the host tissues must be accomplished through the anther walls. Although these anther-wall infections have not been followed through successive stages, appressoria produced from the mycelium in the inter-anther spaces have been observed on the anther walls, and some mycelial penetration at such points has been noticed. From this stage fungous invasion of the anther tissues is very rapid. The flower buds lie so closely together in the axillary buds that usually all of the flowers from a given bud are infected. All of the anthers of an infected flower may be infected (Fig. 1).

## CONTROL

During the last 3 seasons, sprays have been applied empirically for the control of stamen blight of Young dewberry, since the critical infection period was not certainly known. In 1939 and 1940, lime-sulphur, Bordeaux mixture, and a spray known as Truex (containing ammonium polysulphides) were applied at various strengths in January, February, and March. None of the sprays was effective.

Following the knowledge gained concerning the time of infection from experiments of 1941 it is the purpose of the writers to try protective sprays on axillary buds of the young canes from the period of full bloom until the berries are about half-size. However, lime-sulphur (4 per cent of 32° Baumé) applied August 20, 1941, resulted in 58.9 per cent control, as indicated by records taken May 19, 1942.

SUMMARY

A disease of the anthers of blackberry flowers is described. The causal organism, *Hapalosphaeria deformans* Sydow, infects through the anther walls and forms a pseudoparenchymatous envelope completely surrounding the pollen sac. Pycnidia formed on this pseudoparenchyma erupt on the surface ready to discharge coils of pycnidiospores when the flowers open. Infection takes place through the axillary buds sometime between May and March. No perfect stage of the causal fungus has been discovered. Lime-sulphur or Bordeaux mixture applied as a dormant spray in January, February, and March have afforded no control. Lime-sulphur applied in August has given indication of about 60 per cent control. Commercial varieties affected in Oregon are Boysen and Young dewberries and Evergreen blackberry.

OREGON AGRICULTURAL EXPERIMENT STATION,
CORVALLIS, OREGON.

LITERATURE CITED

1. SYDOW, H., and P. SYDOW. Mycotheca germanica. Fasc. XII–XIII. Ann. Myc. 5: 395–399. 1907.
2. DIEDICKE, H., and H. SYDOW. Über *Paepalopsis deformans* Syd. Ann. Myc. 6: 301–305. 1908.
3. DEARNESS, J., and W. R. FOSTER. Anther and stigma blight of Loganberry. Canad. Jour. Res. 9: 43. 1933.
4. GROVES, W. B. British stem- and leaf-fungi. 1: 130. Univ. Press (Cambridge). 1935.
5. ZELLER, S. M. Stamen blight of blackberry caused by *Hapalosphaeria deformans*. (Abstract) Phytopath. 29: 829. 1939.

# MYCORHIZAE ASSOCIATED WITH SOME COLORADO FLORA

W. D. THOMAS, JR.[1]

(Accepted for publication May 30, 1942)

In order to obtain more information concerning the mycorhizae of the Rocky Mountain region, a study was made to determine the types of mycorhizae associated with certain Colorado flora.

According to McDougall and Jacobs,[2] "Mycorhizas are not so abundant in the central Rocky Mountain forests as in some of the deciduous forests of the east. . . ." These workers found only endotrophic mycorhizae on *Pinus contorta, Juniperus monosperma,* and on three species of *Cercocarpus.* They found the ectendotrophic forms on *Pseudotsuga taxifolia* and *Populus tremuloides,* and both ectotrophic and endotrophic types on *Picea engelmanii.* Ectotrophic forms alone were found on *Abies lasiocarpa* and *Pinus flexilis.*

## METHODS AND MATERIALS

The materials used in this study were collected in central and northern Colorado in the autumn of 1939 and the spring and summer of 1940. Most of the material was taken before the ground had become frozen; however, in the higher mountain regions some of the material had to be taken from frozen ground.

The roots of the specimens were taken in their entirety whenever possible. When the specimens were taken from frozen ground the entire root system was taken up with its surrounding soil and then thawed in the laboratory. The roots were killed and fixed in standard Formalin-acetic-alcohol solution, cleared, and embedded in paraffin, according to standard procedure. Sections were then stained with safranin and fast-green.

All mycorhizae found in this study were classified under the following types:

1. Endotrophic[3] (Fig. 1)
   a. Peloton—coiled masses of hyphae in the cells.
   b. Arbuscule—branched haustoria.
   c. Vesicle—"probably abortive sporangia."
2. Ectotrophic[4]
   a. Coralloid—the short-roots becoming dichotomously branched.
   b. Ball—a short, branching growth resulting in a ball composed of rootlets bound together by hyphae.

[1] The writer gratefully acknowledges his indebtedness to Dr. L. W. Durrell for suggesting this work and for helpful suggestions during the progress of the investigations. Thanks are due also to Dr. W. A. Kreutzer, Colorado State College, Dr. E. C. Stakman and Dr. C. M. Christensen, University of Minnesota, for reading and criticizing the manuscript, and to Dolores B. Thomas for assistance in preparing the camera-lucida diagrams.

[2] McDougall, W. B., and M. C. Jacobs. Tree mycorhizas of the Central Rocky Mountain region. Amer. Jour. Bot. 14: 258–266. 1927.

[3] Burges, Alan. On the significance of mycorrhiza. New Phytol. 35: 117–129. 1936.

[4] Laing, E. V. Studies on tree roots. Forestry Comm. Bull. 13. London. 1932.

3. Ectendotrophic—a combination of endotrophic and ectotrophic types.[5]
4. Pseudomycorhiza.

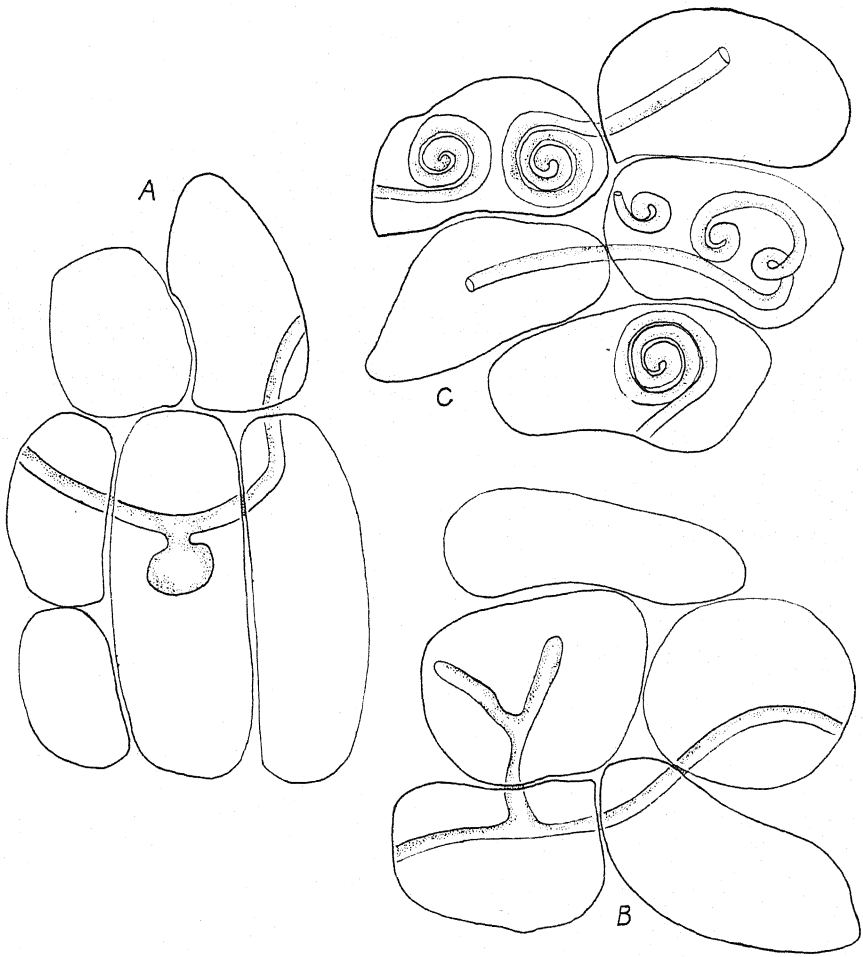



FIG. 1. Endotrophic mycorhizae. A. Vesicle in *Cirsium drumondii*. B. Arbuscule in *Potentilla gracilis*. C. Peloton in *Helianthella quinquinerva*. (All drawings made with the aid of a camera lucida.)

## EXPERIMENTAL RESULTS

Ectotrophic mycorhizae appeared only on trees and shrubs, the coralloid type predominating. The ball type, with the exception of those found on *Salix scouleriana*, appeared exclusively on the members of the Betulaceae (Table 1).

Ectendotrophic mycorhizae were rare, appearing only on the roots of trees and shrubs. This type was found in only 6 species: *Picea engelmanii*,

[5] Although there is some doubt as to the ability of one fungus to produce ectendotrophic mycorhizae, this type was considered separately for convenience.

TABLE 1.—*Types of mycorhizae found on Colorado flora*

| Family and specimen | Endotrophic | | | Ectotrophic | | Etendotrophic | Pseudomycorhiza |
|---|---|---|---|---|---|---|---|
| | Peloton | Arbuscule | Vesicle | Coralloid | Ball | | |
| *Pinaceae* | | | | | | | |
| *Pinus contorta* Loud. | − | − | − | + | − | − | + |
| *flexilis* James | − | − | − | + | − | − | + |
| *ponderosa* Laws. | − | − | − | + | − | − | + |
| *Picea pungens* Engelm. | − | − | − | + | − | − | + |
| *engelmanii* Engelm. | − | − | − | − | − | + | + |
| *Pseudotsuga taxifolia* Britt. | − | − | − | + | − | − | + |
| *Abies concolor* Lindl. & Gord. | − | − | − | + | − | − | − |
| *Juniperus scopulorum* Sarg. | − | − | − | + | − | − | + |
| *virginiana* L. | − | − | − | + | − | − | + |
| *communis* L. | − | − | − | + | − | − | − |
| *Salicaceae* | | | | | | | |
| *Populus deltoides* L. | − | − | − | + | − | − | − |
| *tremuloides* Michx. | − | − | − | + | − | − | − |
| *angustifolia* James | − | − | − | − | − | − | − |
| *Salix scouleriana* Barr. | − | − | − | − | + | − | − |
| *Fagaceae* | | | | | | | |
| *Quercus utahensis* Rydb. | − | − | − | + | − | − | − |
| *Ulmaceae* | | | | | | | |
| *Ulmus americana* L. | − | − | − | + | − | − | + |
| *Celtis occidentalis* L. | − | − | − | + | − | − | − |
| *Rosaceae* | | | | | | | |
| *Prunus americana* Marsh. | − | − | − | + | − | − | + |
| *pennsylvanica* L. | − | − | − | + | − | − | + |
| *virginiana* L. | − | − | − | + | − | − | − |
| *Cercocarpus montanus* Raf. | − | − | − | − | − | + | − |
| *Purshia tridentata* DC. | + | − | − | − | − | − | − |
| *Potentilla effusa* Dougl. | − | − | + | − | − | − | − |
| *fruticosa* L. | − | − | + | − | − | − | − |
| *gracilis* Dougl. | − | + | + | − | − | − | − |
| *Sorbus scopulina* Britt. | − | − | − | − | − | − | − |
| *Physocarpus monogynus* (Torr.) A. Nels. | − | − | − | − | − | − | − |
| *Rubus deliciosus* James | − | − | − | − | − | − | − |
| *Fragaria ovalis glauca* (Wats.) A. Nels. | − | − | − | − | − | − | − |
| *Geum rivale* L. | − | − | − | − | − | − | − |
| *Rosa woodsii* Lindl. | − | − | − | − | − | − | − |
| *Betulaceae* | | | | | | | |
| *Betula fontinalis* Sarg. | − | − | − | − | + | − | − |
| *Alnus tenuifolia* Nutt. | − | − | − | − | + | − | − |
| *Oleaceae* | | | | | | | |
| *Fraxinus pennsylvanica* Marsh. | − | − | − | − | − | + | − |
| *Aceraceae* | | | | | | | |
| *Acer glabrum* Torr. | − | − | − | − | − | − | − |
| *Leguminosae* | | | | | | | |
| *Robinia pseudoacacia* L. | − | − | − | − | − | + | − |
| *Gleditsia triacanthos* L. | − | − | − | − | − | + | + |
| *Thermopsis divaricarpa* A. Nels. | + | − | − | − | − | − | − |
| *Medicago sativa* L. | − | − | + | − | − | − | − |
| *Lupinus parviflorus* Nutt. | − | − | − | − | − | − | − |
| *Astragalus parryi* Gray | − | − | − | − | − | − | − |
| *Oxytropis saximontana* A. Nels. | − | − | − | − | − | − | − |
| *Polygonaceae* | | | | | | | |
| *Polygonum bistortoides* Pursh. | + | − | + | − | − | − | − |
| *Eriogonum alatum* Torr. | − | − | − | − | − | − | − |
| *umbellatum* Torr. | − | − | − | − | − | − | − |
| *Oxyria digyna* (L.) Camptd. | − | − | − | − | − | − | − |

TABLE 1.—(*Continued*)

| Family and Specimen | Endotrophic | | | Ectotrophic | | Ectendotrophic | Pseudomycorhiza |
|---|---|---|---|---|---|---|---|
| | Peloton | Arbuscule | Vesicle | Coralloid | Ball | | |
| *Polemoniaceae* | | | | | | | |
| *Polemonium viscosum* Nutt. | + | – | – | – | – | – | – |
| *Gentianaceae* | | | | | | | |
| *Gentiana elegans* A. Nels. | + | – | – | – | – | – | – |
| *parryi* Engelm. | + | – | – | – | – | – | – |
| *Swertia palustris* A. Nels. | + | – | – | – | – | – | – |
| *Frasera speciosa* Griseb. | – | – | + | – | – | – | – |
| *Scrophulariaceae* | | | | | | | |
| *Pedicularis groenlandica* Retz. | + | – | + | – | – | – | – |
| *Pentstemon secundiflorus* Benth. | + | – | – | – | – | – | – |
| *Pyrolaceae* | | | | | | | |
| *Moneses uniflora* (L.) Gray | + | – | – | – | – | – | – |
| *Primulaceae* | | | | | | | |
| *Dodecatheon pauciflorum* (Durand) Greene | + | – | – | – | – | – | – |
| *Labiatae* | | | | | | | |
| *Monarda menthaefolia* Grah. | + | – | – | – | – | – | – |
| *Saxifragaceae* | | | | | | | |
| *Saxifraga rhomboidea* Greene | + | – | – | – | – | – | – |
| *Heuchera bracteata* Torr. | – | – | – | – | – | – | – |
| *Ranunculaceae* | | | | | | | |
| *Aconitum columbianum* Nutt. | + | – | – | – | – | – | – |
| *Delphinium subalpinum* (Gray) A. Nels. | + | – | – | – | – | – | – |
| *Pulsatilla hirtissima* (Pursh.) Britt. | + | – | + | – | – | – | – |
| *Actaea arguta* Nutt. | – | – | + | – | – | – | – |
| *Ranunculus adoneus* Gray | – | – | – | – | – | – | – |
| *Orchidaceae* | | | | | | | |
| *Limnorchis stricta* (Lindl.) Rydb. | + | – | – | – | – | – | – |
| *Corallorhiza multiflora* Nutt. | – | + | – | – | – | – | – |
| *Spiranthes stricta* (Rydb.) A. Nels. | – | – | – | – | – | – | – |
| *Convallariaceae* | | | | | | | |
| *Streptopus amplexifolius* (L.) DC. | – | – | – | – | – | – | – |
| *Smilacina stellata* (L.) Desf. | – | – | – | – | – | – | – |
| *Onagraceae* | | | | | | | |
| *Epilobium angustifolium* L. | – | – | – | – | – | – | – |
| *Oenothera nuttalli* Sweet | – | – | – | – | – | – | – |
| *Crassulaceae* | | | | | | | |
| *Sedum stenopetalum* Pursh. | – | – | – | – | – | – | – |
| *Papaveraceae* | | | | | | | |
| *Corydalis aurea* Willd. | – | – | – | – | – | – | – |
| *Umbelliferae* | | | | | | | |
| *Angelica ampla* A. Nels. | – | – | – | – | – | – | – |
| *Hydrophyllaceae* | | | | | | | |
| *Phacelia sericea* (Graham) Gray | – | – | – | – | – | – | – |
| *Melanthaceae* | | | | | | | |
| *Zygadenus elegans* Pursh. | – | – | – | – | – | – | – |
| *Liliaceae* | | | | | | | |
| *Allium geyeri* Wats. | – | – | – | – | – | – | – |
| *Leucocrinum montanum* Nutt. | – | – | – | – | – | – | – |
| *Lilium montanum* A. Nels. | – | – | – | – | – | – | – |
| *Liliaceae* | | | | | | | |
| *Erythronium parviflorum* (Wats.) L. | – | – | – | – | – | – | – |
| *Yucca glauca* Nutt. | – | – | – | – | – | – | – |
| *Calochortus gunnisonii* Wats. | – | – | – | – | – | – | – |
| *Iridaceae* | | | | | | | |
| *Iris missouriensis* Nutt. | – | – | – | – | – | – | – |

TABLE 1.—(*Continued*)

| Family and Specimen | Endotrophic | | | Ectotrophic | | Ectendotrophic | Pseudomycorhiza |
|---|---|---|---|---|---|---|---|
| | Peloton | Arbuscule | Vesicle | Coralloid | Ball | | |
| *Cruciferae* | | | | | | | |
| *Erysimum asperum* DC. | – | – | – | – | – | – | – |
| *Ericaceae* | | | | | | | |
| *Arctostaphylos uva ursi* (L.) Spreng. | – | – | + | – | – | – | – |
| *Berberidaceae* | | | | | | | |
| *Berberis aquifolium* Pursh. | – | – | – | – | – | – | – |
| *Grossulariaceae* | | | | | | | |
| *Ribes saximontanum* E. Nels. | – | – | + | – | – | – | – |
| *Hydrangeaceae* | | | | | | | |
| *Jamesia americana* T. & G. | – | – | – | – | – | – | – |
| *Campanulaceae* | | | | | | | |
| *Campanula parryi* Gray | – | – | + | – | – | – | – |
| *Geraniaceae* | | | | | | | |
| *Geranium richardsonii* F. & M. | – | – | – | – | – | – | – |
| *Monotropaceae* | | | | | | | |
| *Pterospora andromeda* Nutt. | – | + | – | – | – | – | – |
| *Vacciniaceae* | | | | | | | |
| *Vaccinium scoparium* Leib. | – | – | + | – | – | – | – |
| *oreophilum* Rydb. | – | – | + | – | – | – | – |
| *Chenopodiaceae* | | | | | | | |
| *Blitum capitatum* L. | – | + | – | – | – | – | – |
| *Boraginaceae* | | | | | | | |
| *Oreocarya virgata* (Porter) Greene | – | – | + | – | – | – | – |
| *Caprifoliaceae* | | | | | | | |
| *Sambucus microbotrys* Rydb. | – | + | – | – | – | – | – |
| *Linnaea americana* Forbes | – | – | + | – | – | – | – |
| *Compositae* | | | | | | | |
| *Helianthella quinquinervis* (Hook.) Gray | + | – | – | – | – | – | – |
| *Rudbeckia laciniata* L. | + | – | – | – | – | – | – |
| *hirta* L. | – | + | – | – | – | – | – |
| *Compositae* | | | | | | | |
| *Senecio crassulus* Gray | – | – | – | – | – | – | – |
| *cernuus* Gray | + | – | – | – | – | – | – |
| *eremophilus* Rich. | – | – | – | – | – | – | – |
| *Taraxacum officinale* Weber | – | – | – | – | – | – | – |
| *Chrysothamnus graveolens* (Nutt.) Greene | + | – | – | – | – | – | – |
| *Cirsium drummondii* T. & G. | – | + | + | – | – | – | – |
| *Artemisia frigida* Willd. | – | – | – | – | – | – | – |
| *Antennaria rosea* (Eat.) Gr. | – | – | – | – | – | – | – |
| *Anaphalis subalpina* (Gray) Rydb. | – | – | – | – | – | – | – |
| *Erigeron salsuginosus* Gray | – | – | – | – | – | – | – |
| *macranthus* Nutt. | – | – | – | – | – | – | – |
| *Aster engelmanii* Gray | – | – | – | – | – | – | – |
| *porteri* Gray | – | – | – | – | – | – | – |
| *Gaillardia aristata* Pursh. | – | – | – | – | – | – | – |
| *Machaeranthera varians* Greene | – | – | – | – | – | – | – |
| *Rydbergia grandiflora* (Pursh.) Greene | – | – | – | – | – | – | – |
| *Achillea millefolium* L. | – | – | – | – | – | – | – |

*Prunus virginiana, Fraxinus pennsylvanica, Robinia pseudoacacia, Gleditsia triacanthos,* and *Cercocarpus montanus.* It may be observed that only two of these species are widely separated from the taxonomic standpoint, whereas *Prunus* and *Cercocarpus* are in the same family, as also are *Robinia*

and *Gleditsia*. It also is interesting to note that this type of mycorhiza was not found in other members of *Picea* or *Prunus* then under observation.

Among the endotrophic mycorhizae observed, the peloton type was the most common, being the only type found in the Polemoniaceae, Pyrolaceae, Primulaceae, Labiatae, and the Saxifragaceae. The vesicular type also was common, occurring in the Salicaceae, Leguminosae, Polygonaceae, Gentianaceae, Ranunculaceae, Ericaceae, Grossulariaceae, Campanulaceae, Vacciniaceae, Caprifoliaceae, and Compositae. It may be noted that this type was consistent in its occurrence among generic groups, but was relatively inconsistent in its occurrence among family groups.

The arbuscular type, however, was somewhat rare. Arbuscules were found in *Potentilla gracilis, Corallorhiza multiflora, Pterospora andromeda, Blitum capitatum, Sambucus microbotrys, Rudbeckia hirta,* and *Cirsium drummondii*. It may be noticed that only in the case of *Rudbeckia hirta* and *Cirsium drummondii* were arbuscules found twice in the same family. A study of the occurrence of these various endotrophic types shows that more than one type of endotrophic mycorhiza may appear in a certain species, as was the case in *Potentilla gracilis, Polygonum bistortoides, Pedicularis groenlandica,* and *Cirsium drummondii*. No true endotrophic mycorhizae were found in the roots of trees or shrubs.

Another interesting observation was that pseudomycorhizae occurred almost exclusively on the Pinaceae, with the exception of those found on *Ulmus americana, Prunus americana, P. pennsylvanica, Acer glabrum,* and *Gleditsia triacanthos*.

The presence of a humus soil did not seem to control altogether the formation of mycorhizae, for mycorhizae were found on *Pinus ponderosa, Frasera speciosa,* and *Cirsium drummondii,* occurring in sandy soils.

It was interesting to note that all mycorhizae observed, with the exception of those in the Orchidaceae, were found among the gymnosperms and the dicotyledons. Mycorhizas were found from 5,000 to 11,500 feet above sea level, being most abundant between the elevations of 7,500 to 10,500 feet.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY,
COLORADO STATE COLLEGE,
FT. COLLINS, COLORADO.

# LIGHTNING INJURY TO COTTON[1]

A. L. SMITH

(Accepted for publication June 2, 1942)

Small areas showing lightning-injured cotton plants may be found throughout Georgia from May to September. Since 1936 the writer has been requested to examine a number of cotton fields where the growers were unable to diagnose the cause of the damage. Careful observation has indicated that such diagnostic difficulties are due to the wide variations in symptoms. These, from the standpoint of field appearance, may be grouped under two general types: (1) Well-defined, circular areas of dead plants that appear immediately or within a few days after an electrical storm. These areas have a marginal intermingling of living and dead plants (Fig. 1, A). (2) Of less frequent occurrence is a dispersed-delayed type of injury in which none or very few plants are killed outright. Usually no evidence of injury appears until ten days or more following the electrical storm. Scattered plants turn red, wilt and die (Fig. 1, B). The delayed appearance of symptoms and the absence of a well-defined central point does not readily suggest the possibility of lightning injury. Innumerable gradations between these two general types of field symptoms are encountered.

The condition that invariably appears to be associated with centralized killings is high soil moisture extending beyond the surface layer. Apparently surface water may or may not be present. High soil moisture apparently aids downward conduction of the charge and consequent killing of the underground stem, tap root, and larger lateral roots. This is followed by the sudden wilting of the above-ground parts. The downward rather than outward movement of the charge also limits the size of areas affected. The killed areas vary from 15 to 50 feet in diameter, with killed plants intermingled with the living for some distance beyond. The magnitude of the discharge obviously determines the size of the areas affected when soil resistances are similar. Areas are often elongated in the direction of the rows. The alternate furrows and ridges might result in alternate high and low soil moisture levels or streams of surface water that would tend to influence movement of the electricity along the rows. Young plants are killed outright more readily than older ones, probably because of the smaller amount of woody tissue and the nearness of the root system to the soil surface. Whipple (7) observed that higher percentages of young tomato plants were killed than of older ones. These outright killings in circular spots usually are correctly diagnosed. Consequently, the present article will be confined to the injuries that are more difficult to diagnose.

[1] Cooperative investigations between the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and the Georgia Experiment Station, Experiment, Georgia. Paper No. 95, Journal Series, Georgia Experiment Station.

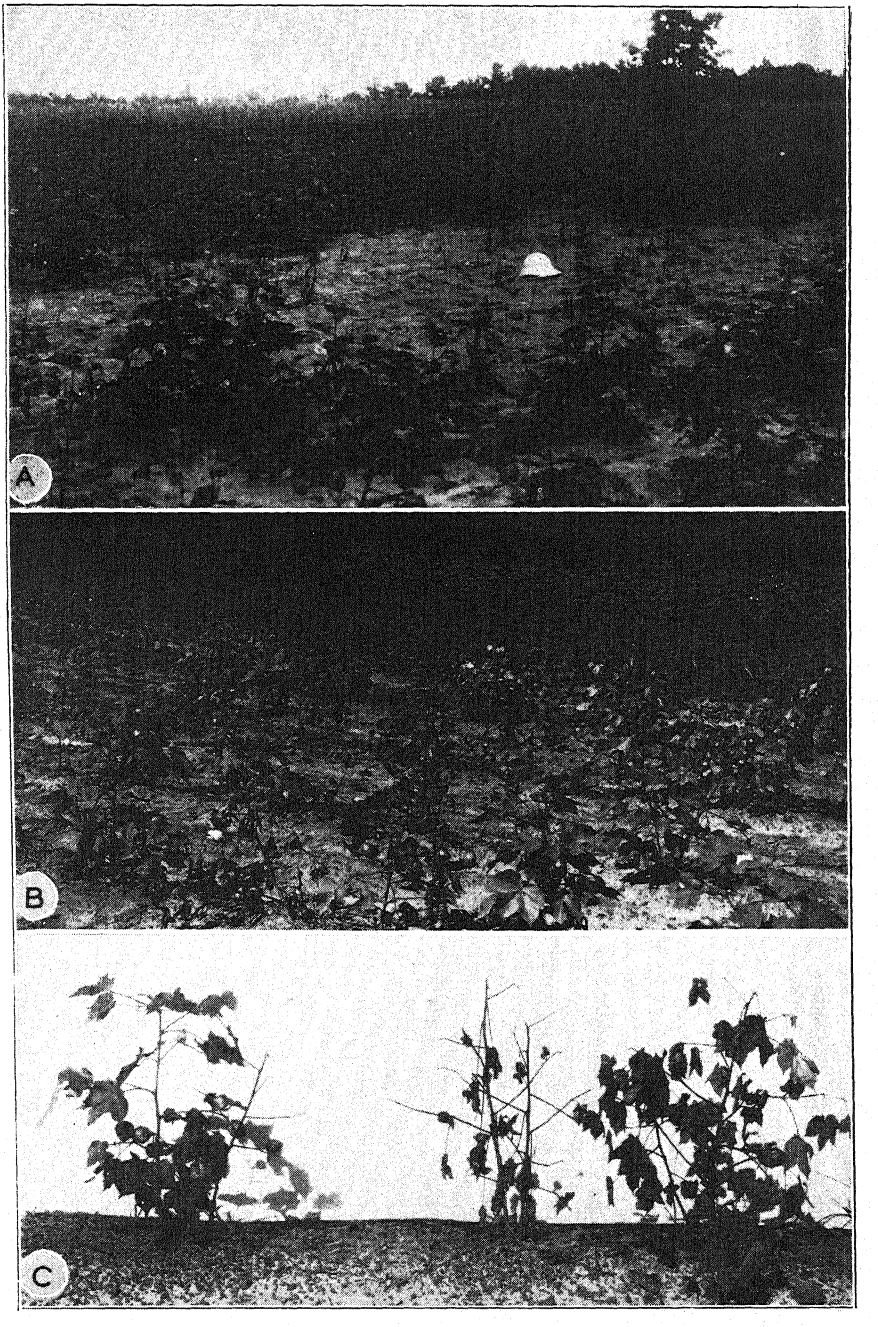



FIG. 1. Lightning-struck cotton fields. A. Typical lightning-struck spot showing denuded center with marginal killing. B. Dispersed type of injury with dead, wilting, and unaffected plants intermingled. C. Portion of cotton row in dispersed area with a dead and living plant in the same hill and adjacent hill with both plants killed.

The dispersed-delayed appearance of symptoms was called to the writer's attention in 1936, when specimens from an area approximately 200 feet in diameter were submitted to the writer by H. W. Rankin.[2] The somewhat irregularly distributed wilting and killing of plants over such a large area results in generally roughly circular spots that show no tendency to elongate with the rows. The largest spot so far observed by the writer was about 300 feet wide. Entire hills, or one or more plants from thickly clustered hills, may be affected. A typical portion of a row is shown in figure 1, C. The center of such areas usually can be determined by the higher percentage of plants showing injury; however, no well-defined center may be established for some of the larger areas. Such spots apparently result from lightning discharges that occur in relatively dry soils or in those having a thin moist surface layer with drier soil beneath. Jones and Gilbert (2) state that surface dispersion of a discharge would be expected when the first rain wets the surface soil following a period of dry weather. Where the dispersal has been so limited that a distinct "center" is discernible, diagnosis is less difficult. Plants in the immediate center of struck spots may show mechanical disorganization. Others appear to have been killed immediately through collapse of the cambium over a large portion of the plant, particularly the underground portions including the lower stem, tap root, and primary lateral roots. The pith, especially in small plants, frequently shows disorganization. This is particularly noticeable from the soil line downward; however, in the lower part of the stem the amount of pith may be so small that this character is of little diagnostic value. Further from the center direct injury to the aboveground part of the stem is infrequent. The xylem, except where actual mechanical disorganization of the plant occurs, appears unimpaired and may continue to transmit water to the upper part, even though collapse of the tissues exterior to the xylem completely girdles the stem.

The most characteristic symptom of lightning-injured plants in the delayed-dispersed type spots, and also at the periphery of those showing centralized killing, results from the passage of the charge through the soil near the surface. This produces a girdling of the stem just below the soil line and a consequent enlargement just above this girdle (Fig. 2, A). The first symptom noticeable in such plants is the reddening of the foliage. Developments subsequent to girdling are: Callus formation to produce collar-like enlargements just above the dead tissue; longitudinal or, less frequently, horizontal cracking of the dead cortical tissues; adventitious root development when the proliferated portion is in contact with moist soil; fungus invasion of the xylem at the girdle and, as invasion progresses downward, the development of discoloration and necrosis; wilting of aboveground parts because of their inadequate water supply; and eventual root starvation and death of the affected plants.

In the aboveground parts the general reddening of the foliage is attrib-

[2] Formerly Extension Plant Pathologist for Georgia.

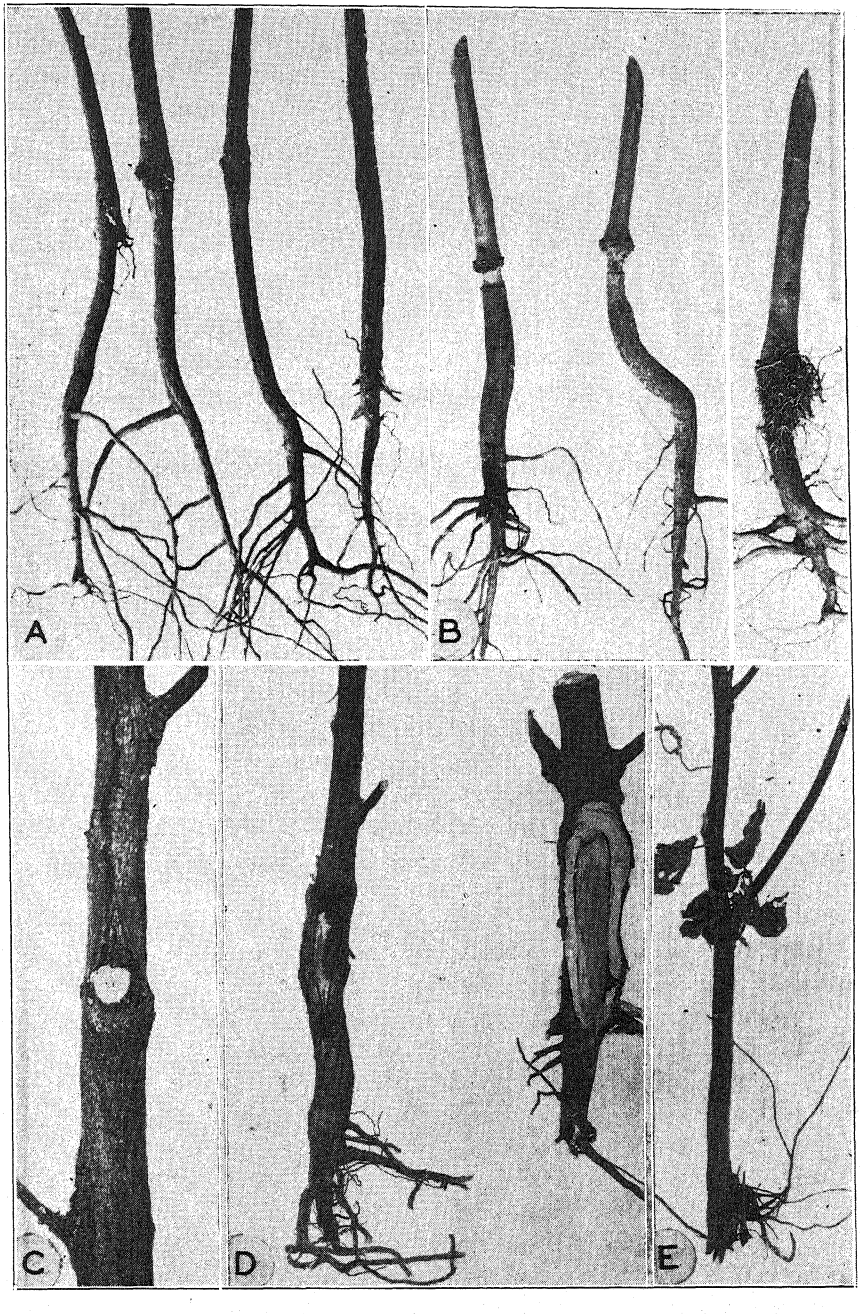



FIG. 2. Lightning-injury symptoms on cotton stems. A. Plants girdled by lightning near the soil line showing a collar-like enlargement above the injury, splitting of the dead cortical tissues, and development of adventitious roots. B. Plants girdled experimentally by removing a band of tissue below the soil line, photographed 28 days after girdling. C. Central stem of cotton plant, sometime after lightning injury, with cracking of the cortex and a wide rupture at the axis of the branch and stem. D. Incompletely girdled cotton stems which survived injury by formation of new xylem tissue when the original xylem became nonfunctional. E. Adventitious-root development sufficient for survival of the plant following complete girdling and decay of the xylem.

uted to accumulated carbohydrates, increased acidity of the cell sap, and anthocyanin pigment development. This symptom usually is followed by rather sudden wilting, death, and defoliation. That the preceding effects were the results of girdling was demonstrated experimentally in the field. Bands of tissue, ½ inch wide and exterior to the xylem, were removed just below the soil line. The resultant symptoms, including enlargements above the girdle, adventitious root development, invasion of the xylem by similar organisms, and the development of anthocyanin pigment (Fig. 2, B), were quite similar to those of lightning-injured plants. The experimentally girdled plants had survived for a period of four weeks when they were removed for examination.

Many variations in symptoms, especially among recovered plants, may be noted. Irregular elongated areas of sunken, necrotic tissue may show on the stems (Fig. 2, C), whereas the lateral branches develop normally. Wide fissures are frequently noticeable above and below the axis of these branches with the stem. The xylem, underlying the affected cortex, continues to conduct water to the uninjured branches; and their growth is affected only slightly, if any. The tops of some plants may be killed down to one of the lateral branches. The pith below the injured cortex in surviving plants is often discolored. The above-described symptoms have been observed at mid-season or later and usually are confined to the more central zone of struck spots. The infrequent occurrence of such symptoms and the irregular patterns of injured tissues suggest that the plants were wet or partly so at the time of lightning discharge.

Continued survival of girdled young plants is apparently not very common. On older plants partial girdling and recovery is more frequent. Recovered plants may appear to be normal. When, however, such plants are pulled and examined, the stems at or under the groundline are flattened, in most instances, due to a new growth of tissues on one side and the exposed xylem on the opposite side. Walker (6) has previously reported greater damage to the side of cabbage stems facing the center of the discharge area. With the plants illustrated in figure 2, D, the cortical and cambial tissues were killed on the side nearest the discharge. The xylem thus exposed became infected by organisms and eventually became non-functional. With other plants an enlargement below the soil line with much cracking of the old cortex was the only evidence of injury having occurred. Occasionally the formation of adventitious roots is adequate for support of the plant after complete decay of the stem at the injury (Fig. 2, E).

The dispersed-delayed lightning injury effect, in some instances, may have been attributed to fungus organisms (1). In fact, in the first instance that came to the writer's attention, pathogenic organisms were suspected, and tissue cultures were made from the xylem of a number of plants. *Rhizoctonia bataticola* (Taub.) Butler was obtained from a majority of the cultures and *R. solani* Kühn was second in frequency of occurrence, often in combination with the former. Other organisms were secured less fre-

quently. It was evident that *R. bataticola* entered the xylem in the region of the girdle and progressed downward into the roots and later invaded the cambium, phloem, and cortical tissues. The tissues above the girdle, supplied with evolved plant food, were not attacked by *R. bataticola,* although a portion of the vessels in this region became discolored. *R. bataticola* is of interest in connection with reports of its pathogenicity on cotton of some species other than *Gossypium hirsutum* in India (4, 5), Greece (3), and Africa (1). American upland varieties in comparison with varieties of *G. herbaceum* were found relatively resistant to this organism (3).

SUMMARY

The general appearance of lightning-struck spots in cotton fields varies considerably. In the more frequently encountered spots sudden killing in small circular areas result. More difficult to diagnose are the somewhat indefinite and variable spots in which dispersed and delayed appearance of symptoms, without noticeable centralized killing, extends over areas as much as 300 feet in diameter. Immediate killing of plants results from the collapse of tissues exterior to the xylem on the stems, tap roots and the larger roots. Plants surviving for varying periods almost invariably show a collar-like enlargement at or just below the soil line where the plants are completely or occasionally partially girdled. The girdling is caused by the killing of cortical and cambial tissues in a band that is usually about ½ inch wide. Other symptoms of surviving plants include irregular, longitudinally-elongated necrotic areas on the side of the stems. *R. bataticola,* reported to be parasitic on certain Asiatic species of cotton, frequently invades the lower stem and roots of lightning-injured plants of *G. hirsutum* in Georgia.

GEORGIA EXPERIMENT STATION,
EXPERIMENT, GEORGIA.

LITERATURE CITED

1. DUCKER, H. C. Third conference on cotton growing problems. Empire Cotton Growing Corporation, (London) pp. 124–125. 1938.
2. JONES, L. R., and W. W. GILBERT. Lightning injury to potato and cotton plants. Phytopath. **5**: 94–102. 1915.
3. SAREJANNI, J. A., and C. B. CORTZAS. Le nature de la resistance du coton au *Macrophomina phaseoli* (Maubl.) Ashby. Reprinted from C. R. Third Congr. int. Path. comp., Vol. 2, pp. 492–494, 1936. (Abs.) Rev. Appl. Mycol. **16**: 249. 1937.
4. SUNDARARAMAN, S. Administration report of the mycologist for the year 1930–31. Madras, 22 pp., 1932. (Abs.) Rev. Appl. Mycol. **11**: 223–224. 1932.
5. VASUDEVA, R. S. Studies on the root-rot disease of cotton in the Punjab. I. Symptoms, incidence, and the cause of the disease. Ind. Jour. Agr. Sci. **5**: 496–512. 1935. (Abs.) Emp. Cotton Grow. Rev. **13**: 159. 1936.
6. WALKER, J. C. Injury to cabbage by lightning. Phytopath. **27**: 858–861. 1937.
7. WHIPPLE, O. C. Injury to tomatoes by lightning. Phytopath. **31**: 1017–1022. 1941.

# DECLINE DISEASE OF RASPBERRY[1]

S. M. Zeller and A. J. Braun

(Accepted for publication May 25, 1942)

A decline disease of the Cuthbert red raspberry has been known to exist in the Pacific Northwest for a number of years. This disease has received rather intensive study, especially in Oregon, and is perhaps the same as the one that has concerned growers of raspberries in British Columbia in recent years.

The disease is manifest wherever the Cuthbert raspberry is grown in western Oregon, and becomes epidemic in districts where this crop is grown intensively, especially where there are large commercial plantings. Our studies on the decline disease have been conducted entirely in Oregon; therefore, the consideration of the occurrence is based on Oregon climatic and edaphic conditions. In some plantings where the disease is just starting the damage is slight, while in others the losses of plants vary from serious to almost total within 3 or 4 years after planting.

The economic importance of the decline disease is measured essentially by the percentage of plants affected. A plant that has had the disease for one full season is almost valueless. Relatively large diseased areas in a planting, therefore, may easily reduce yields below a profitable basis.

Although other varieties and species of *Rubus* show somewhat similar dying out of plants, the decline disease has not been proved outside of the Cuthbert raspberry.

## SYMPTOMS

Unfortunately, there are no consistent or continuous symptoms by which alone the decline disease may be identified. Diagnosis depends more upon chronological performances than upon concrete symptoms. This is truly a "running-out" disease.

If infection takes place late in the season the first indication of the disease the following spring is the retarded appearance of the new succulent shoots. These have more of a reddish color than the earlier healthy canes. During the growing season, however, the leaves show no abnormalities until growth slows down in the autumn. Leaves of diseased plants produced after this time have considerable downward leaf roll and more definite fluting along the veins as compared with the flatter leaf blades on normal plants (Fig. 1). These rolled leaves toward the tips of affected canes possess somewhat less interveinal greenness than normal leaves, and are slightly bronzed along the margins and crests between the veins. The internodes in these tip areas are foreshortened, as indicated in figure 1. This condition, as suggested above, usually does not occur at other times during the growing season, under field conditions. In the greenhouse, however, this leaf-rolled condition is a predominant symptom throughout the cane-growth period

[1] Published as Technical Paper No. 411, with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany.

(Fig. 3, A). Canes of affected plants, under field conditions, do not attain the height and diameter of normal growth and are otherwise weakened and unhardy, as indicated by the fact that they may die in the winter, or the buds lack the vitality to produce lateral growth the following spring. A general decrease in size and abundance of the root system parallels the depletion of the canes. The smaller roots and feeder rootlets gradually become less numerous as the disease progresses. The influence of all of these

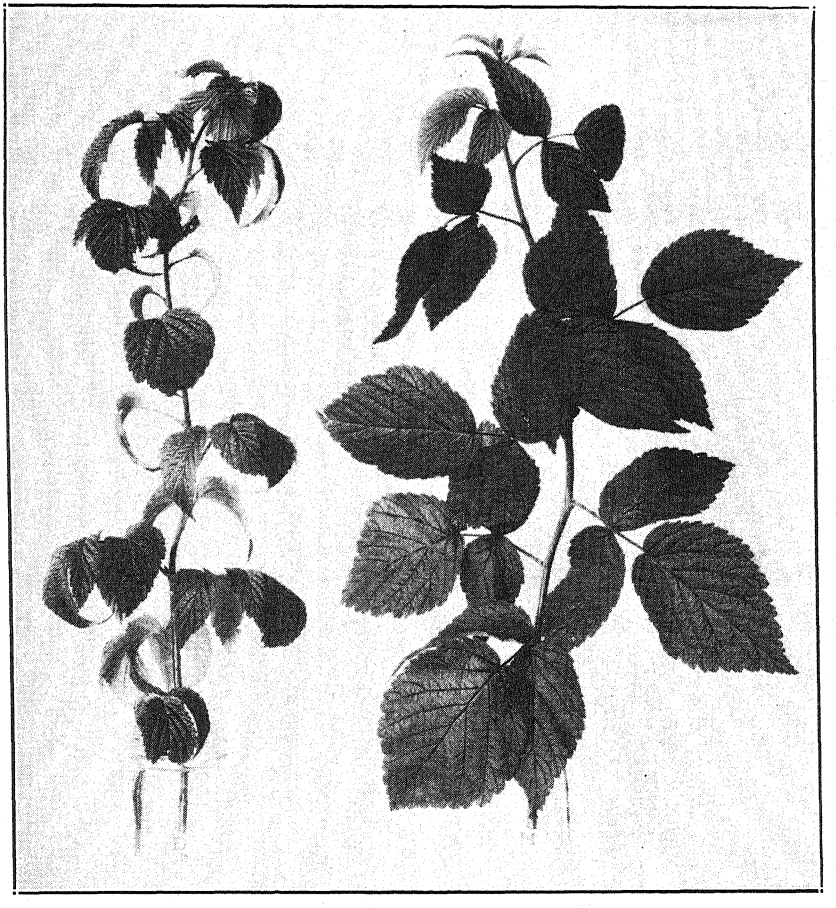


FIG. 1. Tips of current season growth of Cuthbert raspberry canes taken from the field, October 8, 1941. Cane on left infected by decline disease shows leaf rolling and fluting symptoms. Healthy cane at right.

retarding factors results in a progressive deterioration of the whole plant over a maximum of about 3 years (Fig. 3, B).

The raspberry decline disease has no leaf or cane symptoms similar to those that characterize other described virous diseases, but fruit symptoms are similar to those of the Cuthbert raspberry infected with green-mottle mosaic (Rubus virus 1) or yellow mosaic (Rubus virus 2). The fruits are

small, irregular, and tend to be globose rather than ovoid. When the ripe berries are picked the druplets fall apart readily, producing a condition commonly referred to as crumbliness. The berries, therefore, are worthless, and are not harvested from infected portions of the fields.

The disease usually forms circular areas by spreading from foci, which doubtless are single infected plants. The effects of the disease in this respect are quite similar to those resulting from root-weevil infestation. Figure 2 illustrates one of these areas, which was over 200 feet in diameter. There are usually no healthy plants left within the involved areas. This "clean

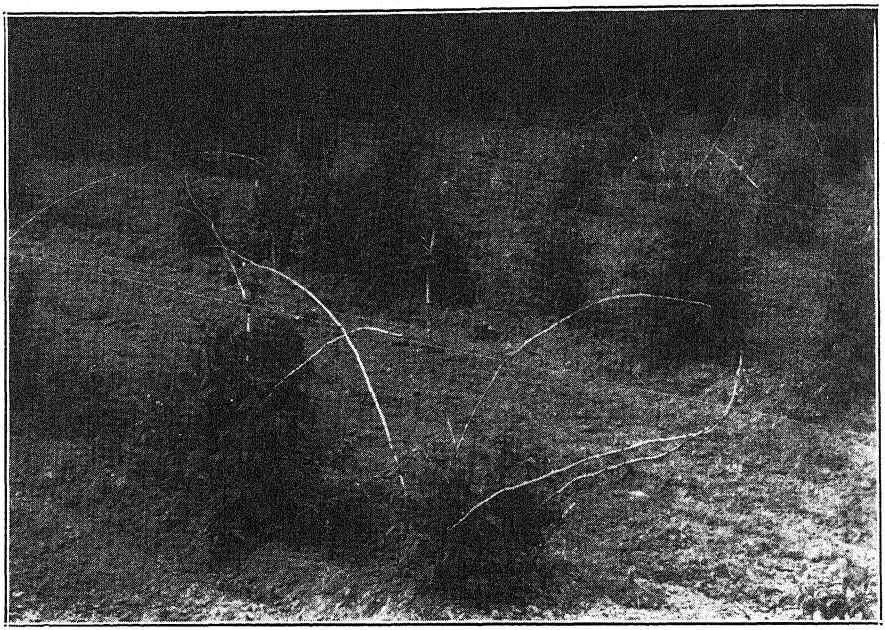


FIG. 2. Sector of a diseased area in Cuthbert raspberry planting showing damage done by the decline disease. (Photographed May 25, 1940.)

sweep" by the disease might lead to the conjecture that spread of infection involves some underground factor, such as natural root grafts or a hypogeous biotic vector. Decline disease occurs on several different soils, indicating that type of soil is probably not a factor in its epidemiology. Drainage and fertility are not factors in all localities where the disease occurs.

## EXPERIMENTAL STUDIES

Previous to the grafting experiments that resulted in the transfer of the disease from affected buds or patches of bark to healthy Cuthbert raspberry plants, many types of empirical tests were made to learn something of the nature of the disease. Following unsuccessful attempts to isolate a causal organism from canes and roots, samples of soil from diseased areas were cultured by W. B. Bollen, Soil Bacteriologist, Oregon State College. No

unusual soil or parasitic organism was found. Healthy and diseased plants were grown in pots in the greenhouse in soils originating from healthy areas in the field where the decline disease was prevalent. In all such cases

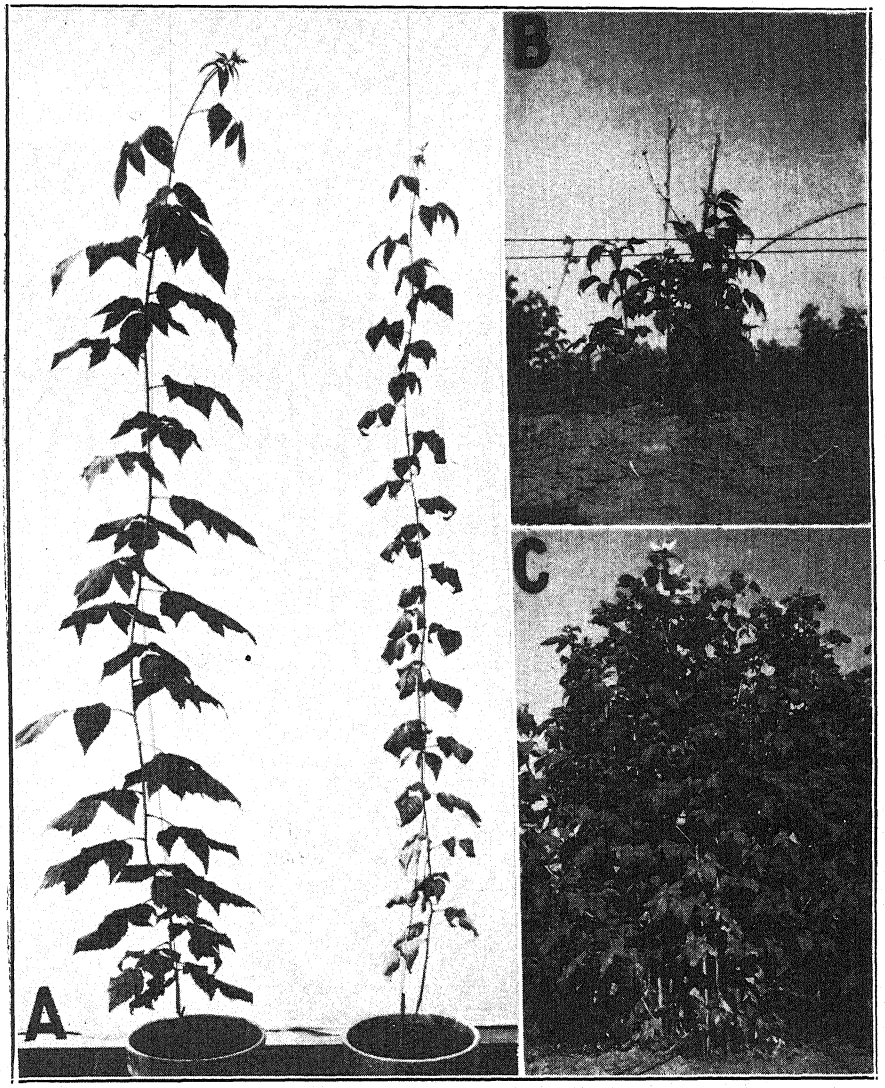



FIG. 3. A. Cuthbert raspberry plants grown in greenhouse: One at right infected by decline disease shows extreme leaf symptoms; one at left, healthy. (August 5, 1941.) B. Diseased plant photographed in the field. C. Healthy plant in same row and 50 feet from diseased plant B. (June 26, 1941.)

the healthy plants remained healthy and diseased plants developed acute symptoms (Fig. 3, A).

When a healthy and a diseased plant were grown in a single pot of soil, the healthy plant in each case has remained healthy for one year, even though

the plants became pot-bound by the intermingling of the roots of the two plants. Negative results from several such experiments indicate that natural grafts of raspberry roots in close proximity in the soil are not readily accomplished.

Good balanced fertilizers, or stable manure, added to the culture of plants with the decline disease in the field greatly stimulated their growth, but did not effect a cure or result in the eradication of the disease.

## TRANSMISSION BY GRAFTING

Although under field conditions there is very little about the disease to suggest a virous etiology, we finally resorted to the grafting of roots and canes. In several cases roots of healthy plants were grafted to roots of diseased plants, buds from diseased canes were grafted into canes of healthy plants, and diseased plants were inarch-grafted to healthy plants. So far no cases of transmission through root grafts have been apparent, but in all cane-graft experiments, whether organic union between diseased cions and healthy canes was or was not accomplished, the disease was transmitted to the healthy plants. In some cases of bud grafts the diseased buds did not live, but the disease was transmitted to the healthy plants, as through "patch" grafts. All of the above grafts were made in late summer on potted plants in the greenhouse. No symptoms appeared that autumn (1941). Accordingly, these potted raspberry plants were set outside all winter and brought back into the greenhouse the next spring (1942). As soon as leaves were well developed the leaf-rolling symptoms of the decline disease characteristic of greenhouse-grown plants appeared throughout the stock plants that had been healthy the previous season. When new succulent canes appeared from the soil they bore rolled leaves, and showed all the characteristics of the disease.

It appears, therefore, that the decline disease is caused by a virus, which is readily transmitted through grafting. A total of 8 cane grafts was made. Transmission of the decline virus of *Rubus* was effected in all.

No insect vector of this virosis has been discovered.

## CONTROL

No specific measures have been tested for the control of the raspberry-decline disease. Incidentally, however, plant selections, made by Rowell Brothers, Scholls, Oregon, to rid Cuthbert raspberry planting stock of a bud-perpetuated disease, known locally as "crumbly berry," have resulted also in a stock that has remained free from the decline disease for several years after planting in disease-free soil. Two Cuthbert plantings within a locality where the decline disease has been epidemic have recently been certified by the Agricultural Extension Service of Oregon State College as free from all known viroses, including that now known as the decline disease. Control of the disease will, therefore, be attempted through certification of planting stock. Other varieties of raspberries and other species of *Rubus* are under test for resistance to decline.

For want of a better descriptive common name for this virosis we are using decline disease of raspberry. As a binomial we suggest **Minuor ruborum** (from *minuor,* to dwindle or decay, and *ruborum,* of *Rubus*) : and Rubus virus 8 in the numerical system.

SUMMARY

A disease of Cuthbert red raspberry has caused the decline of large areas of plants in commercial plantings throughout the Willamette Valley of western Oregon. Affected plants present no particular disease symptoms, except a gradual dwindling in vigor of canes and roots, and some leaf rolling and fluting in late autumn. This leaf roll is characteristic throughout the season when affected plants are grown in the greenhouse.

This decline disease of raspberry has been transmitted by grafting diseased buds onto canes of healthy plants. No insect vector has been discovered. The common name, decline disease of raspberry, is proposed for this virosis, *Minuor ruborum* is suggested as a binomial, and Rubus virus 8 as the numerical designation.

OREGON STATE COLLEGE AGRICULTURAL EXPERIMENT STATION,
CORVALLIS, OREGON.

# SOME EFFECTS OF SAND AND NUTRIENT SUPPLY ON A PHYSIOLOGICAL LEAF SPOT OF CANTALOUPE[1]

E. C. PIERCE AND D. L. STODDARD

(Accepted for publication May 30, 1942)

Attempts to grow cantaloupe (*Cucumis melo* L.), during the winter, in quartz sand with a constant supply of nutrient have not produced plants of desired vigor or quality. Plants so grown were rather spindling and lacked deep green color. The lower leaves developed water-soaked areas, which collapsed within a period of 24 to 36 hours. The leaf spotting, in severe cases, resulted in progressive defoliation of the plant until only the young leaves near the growing point remained.

Unpublished data of Crane and Shear have shown that similar troubles with tung trees grown in sand were apparently caused by lack of aeration because of the large water-holding capacity of the sand and that these troubles might be alleviated by using a coarser sand. Since cantaloupe plants are known to grow best in a well-aerated soil, it was thought that the difficulties experienced in the greenhouse may have resulted from inadequate aeration of the roots or from the method of applying the nutrient solution.

Two grades of white quartz sand were employed (Table 1). The ratio of air space in coarse sand as compared to fine sand was 8 : 1. Four methods of

TABLE 1.—*Sieve size of the two grades of sand used*

| Sieve mesh | Per cent of sand passing through | |
|---|---|---|
| | Course "F" | Fine No. 1 |
| | *Per cent* | *Per cent* |
| 20 | 99.650 | 100.000 |
| 40 | 6.490 | 52.140 |
| 60 | 0.225 | 2.700 |
| 80 | 0.134 | 1.410 |
| 100 | 0.058 | 0.455 |

supplying nutrient solution were used, viz.: (1) 1000 ml. per crock, per 24 hours, drip; (2) 1000 ml. per crock, per 24 hours, slop; (3) 500 ml. per crock, per 24 hours, drip; (4) 500 ml. per crock, per 24 hours, slop. The nutrient solution used was of the following composition: 0.005 M · $Ca(NO_3)_2$, 0.0025 M · $KH_2PO_4$, and 0.0025 M · $MgSO_4$. This solution was supplemented with Fe, Mn, Zn, Cu, and B. Because of numerous cloudy days during mid-winter in this area, supplementary illumination was provided during the day (8 a.m. to 6 p.m.) by six 300-watt bulbs arranged on a rack suspended above the crocks. Twelve seeds of the variety White Seeded Pink Meat were sown in each crock and thinned when the first true leaves had appeared,

[1] Scientific Paper No. A28, Contribution No. 1844, Department of Botany, University of Maryland Agricultural Experiment Station.

leaving two uniform plants per crock. The sand was kept damp until emergence, at which time the nutrient treatments were started. The plants were grown for a period of 6 weeks when the various root, stem, and leaf measurements were obtained.

The data on plant growth showed that plants grown on fine sand were significantly[2] longer in stem growth, but the stems were, at the same time, significantly[2] smaller in diameter than stems from coarse sand plants. The

TABLE 2.—*Effect of sand and treatment on the occurrence of physiological spotting on cantaloupe leaves*

| Treatment | Total number of leaves | Affected | Healthy | "t" value |
|---|---|---|---|---|
| | | *Per cent* | *Per cent* | |
| Coarse sand | 350 | 6.3 | 93.7 | |
| Fine sand | 383 | 13.1 | 86.9 | 3.14[a] |
| Drip culture | 364 | 17.3 | 82.7 | |
| Slop culture | 369 | 4.6 | 95.4 | 5.50[b] |
| 1000 ml. per 24 hours | 371 | 14.0 | 86.0 | |
| 500 ml. per 24 hours | 362 | 5.5 | 94.5 | 3.88[a] |
| Fine sand—drip | 193 | 18.7 | 81.3 | |
| Fine sand—slop | 201 | 7.0 | 93.0 | 3.68[a] |
| Coarse sand—drip | 182 | 10.4 | 89.6 | |
| Coarse sand—slop | 167 | 1.8 | 98.2 | 3.86[a] |
| Fine sand | | | | |
| 1000 ml.—drip | 94 | 24.5 | 75.5 | |
| 1000 ml.—slop | 100 | 13.0 | 87.0 | 2.06 |
| 500 ml.—drip | 99 | 13.1 | 86.9 | |
| 500 ml.—slop | 102 | 1.0 | 99.0 | 3.43[a] |
| Coarse sand | | | | |
| 1000 ml.—drip | 92 | 14.1 | 85.9 | |
| 1000 ml.—slop | 83 | 3.6 | 96.4 | 2.52 |
| 500 ml.—drip | 89 | 6.7 | 93.3 | |
| 500 ml.—slop | 84 | 0.0 | 100.0 | ∞ |
| Fine sand—drip | 193 | 23.8 | 76.2 | |
| Coarse sand—drip | 181 | 6.9 | 93.1 | 4.72[b] |
| Fine sand—slop | 202 | 10.5 | 89.5 | |
| Coarse sand—slop | 167 | 1.8 | 98.2 | 7.07[b] |

[a] Significant at odds of 19: 1.
[b] Significant at odds of 99: 1.

different methods of applying the nutrient solution had no effect on these measurements. In general it can be said that the plants grown in the coarse sand were a deeper green, had thicker leaves, and very closely resembled field-grown plants.

The physiological leaf spotting was significantly less on the plants which were grown on the medium providing better aeration (Table 2). This was true regardless of the comparisons made, whether between sands as a whole, between methods of applying the nutrient solution on the same sand type, or

[2] Differences significant at the one per cent level.

between methods of applying nutrient solution on the two sands (except between 1000 ml. drip and 1000 ml. slop on either fine or coarse sand).

It was concluded that, under the conditions of this experiment, the coarse sand was a superior medium to the fine sand and 500 ml. applied once per 24 hours was superior to the other nutrient applications used.

DEPARTMENT OF BOTANY,
UNIVERSITY OF MARYLAND,
COLLEGE PARK, MD.

## PHYTOPATHOLOGICAL NOTES

*An American Oat Disease Found in Western Anatolia.*—On March 24, 1938, leaf spots on fall-sown oats were observed in the experimental field of the Plant Protection Station at Bornova, near Izmir (Smyrn), Western Anatolia. They were oblong, not sharply limited, and whitish to yellowish with a reddish border (Fig. 1). They were somewhat numerous but hardly enough so to cause much damage in that field.

Microscopic examination disclosed a fungous disease. The spores of the fungus were 2-, rarely 3-septate, hyaline, closely joined in acervuli on each

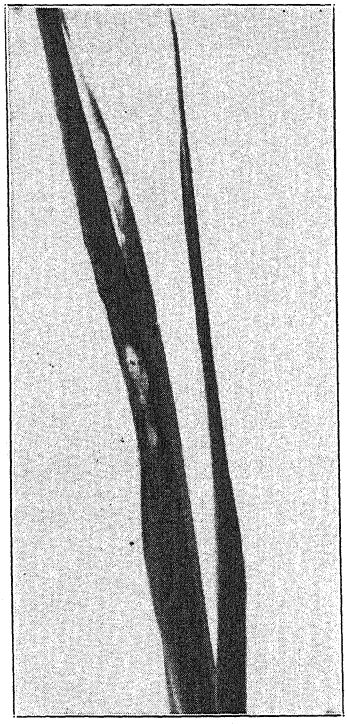


FIG. 1. Leaf spot caused by *Pseudodiscosia avenae.*

side of the leaf. They were fusiform, slightly curved spores, each bearing a single cilium at each end, the one being long and thick, the other short and slender (Fig. 2, left). Thus, they were regarded as belonging to the genus *Pseudodiscosia* Höstermann et Laubert. An end-to-end measurement of 25 conidia, disregarding their curvature, gave the following figures: length, including cilia, 23–45 μ, without cilia, 15–27 μ; average, 34.2 and 20.4 μ, respectively. The maximum width varied from 3 to 4.5 μ, with an average of 3.6 μ. The spores germinated readily in water. The disease has been, until now, unknown in Turkey, and, so far as I know, has not been observed in Europe.

Isolation of the fungus was successfully performed, but its growth was very slow on carrot agar, potato-dextrose agar, and carrot alices. On oatmeal agar and on potato sections there was no growth. In the above cultures the mycelium was hyaline, densely septate, about 3–4 μ in diameter. There was a striking tendency to form conidia of abnormal shape. This tendency was observed in cultures contaminated with bacteria and on pieces of leaves held for some time in a moist chamber, *e.g.*, in the case of increase of accompanying bacteria. The spore cells increased or decreased in number, and were swollen or abnormally slender. The cilia were swollen or their point of insertion changed (Fig. 2, right).

In reading the *Review of Applied Mycology,* I became aware of an American paper on a *Pseudodiscosia* on oats. Writing to the author of this paper, Roderick Sprague, I obtained reprints[1,2,3] that enabled me to identify our oat disease and prove its identity with that first observed in Oregon and

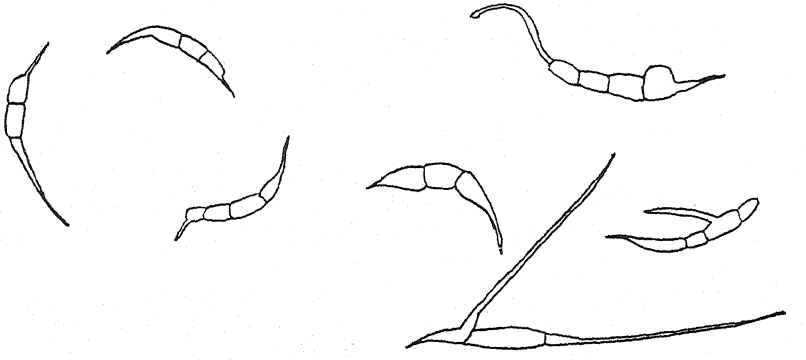


FIG. 2. Conidia of *Pseudodiscosia avenae:* normal (left) ×500; abnormal (right) ×600.

Washington in 1934. The appearance of the disease and the pathogen are the same. The conidia are of the same shape and size (10–42×2–4 μ), according to the American authorities. The slow and scanty development of the fungus on agar also has been observed in America. There, as well as in Turkey, the disease appears in early spring on fall-sown oats only, and disappears after the beginning of April. It seems, therefore, correlated with cool, damp weather. The writer observed it at the experimental field of Bornova in the early spring of 1939 and 1940. To date, it has caused little damage there. The American authors named the pathogen *Pseudodiscosia avenae* Sprague and Johnson.

It is a rather remarkable fact that a fungous disease of such a widegrown and frequently observed crop as oats has been observed till now solely in two countries so remote from each other as are Turkey and the Pacific Northwest of the United States. It seemed, therefore, that it might have

[1] Sprague, R. A new leaf spot on oats. Northw. Sci. 9. 1935.

[2] Sprague, R., and A. G. Johnson. A new *Pseudodiscosia.* Mycologia 28: 181–185. 1936.

[3] Sprague, R. Notes on diseases of cereals and grasses in Oregon and adjacent counties in Washington during the spring of 1938. Pl. Dis. Rptr. 22: 174–175. 1938.

been imported by chance from America to Turkey; the seaport of Izmir, connected by direct steamship lines with North America, is situated not far from the experimental field of Bornova. A further observation, however, makes this assumption rather unlikely. There occurred some leaf spots of the same appearance on a wild grass on a mountain slope at Ören near Kemalpasa, at a distance of about 35 km. from Bornova. In a moist chamber there developed conidia of the same shape and size as those of *Pseudodiscosia avenae:* length, including cilia, 24–39 μ, average of 20 measurements 33.3 μ, without cilia 18–23 μ, the average being 19.9 μ, width 4–5 μ, average 4.25 μ. The grass was not in the flowering stage and, therefore, could not be determined. According to its shape, it might have been probably the wild oat *Avena sterilis* L. which species is to be found very frequently in this region. It seems more likely, therefore, that *Pseudodiscosia avenae* is a native of Turkey, and has either been imported from there to North America, or its area is very much more extended than we are aware of today.—H. BREMER, Güven Sok, No. 35, Kavaklidere, Ankara, Turkey.

*Peach-suture Spot.*—A new peach disease, which seems to affect only the fruit, was found last September in one Elberta orchard on the shore of Lake Ontario in Wayne County, New York. Its striking and distinctive lesions were located exclusively in the suture region, suggesting the name "suture spot" (Fig. 1.) It can be readily distinguished from the red suture

FIG. 1. Symptoms of suture spot on Elberta peach fruits at picking time. The fruit on left has a small sphere-shape lesion in the early, water-soaked stage of development, scarcely visible from the surface. The other fruits show lesions in advanced stages characterized by sunken brownish centers and deep red rims.

virosis[1] which also attacks the same part of the fruit. A similar if not identical disease is known to occur in the Niagara Peninsula of Ontario, Canada where the writer observed it during the autumn of 1940.

The most characteristic position of the lesion is astride the suture or

[1] Cation, D. Three virus diseases of the peach in Michigan. Mich. Agr. Exp. Stat. Circ. Bull. 146: 1–11. 1932.

crease line about midway between the ends of the fruit. Less frequently it may involve only one side of the suture. Only rarely are the spots out of contact with the suture or crease line and when this happens the distance of separation is only a few millimeters.

The spots are ordinarily irregularly oblong ovate in shape but in extreme cases they may be very narrow elongate or almost circular, in surface view. The largest lesions encountered occupied a maximum of one-eighth the circumference midway between the ends of the fruit and ¾ the length of to suture of the fruit. The lesions begin beneath the skin as water-soaked areas tinged with red. Lesion development proceeds first to the surface. In more advanced stages the lesions extend to the pit.

The spot lesions are characteristically red during the earlier or hydrotic stage. The centers soon change to brown upon death of the affected tissues. As the spots enlarge the outer rim becomes red, and remains level with or slightly above the fruit surface, which is in striking contrast to the brown, depressed, dehydrated, or desiccated interior of the lesion. When cut into, the texture of the affected tissues is somewhat corky and tough, with the vascular structure in prominent relief. When affected fruits with spots at different stages of development were examined, following about 6 weeks in cold storage, only some lesions of the earlier stage were found to have enlarged somewhat, and these were on fruits not fully mature.

Suture spot is peculiarly a harvest-time disease. It first attracted attention as the first fruits were beginning to ripen. By the time of the second or final picking certain trees, distributed irregularly through the orchard, had dropped a variable number of the larger more mature fruits to the ground. When it was observed that many of these fruits had striking suture-spot symptoms, it was possible to locate affected trees by looking for trees with fruit on the ground. A few trees had practically every fruit affected. Such trees might be surrounded by trees almost if not completely free of the trouble. Trees with the majority of the drops showing advanced-stage symptoms usually held less mature fruits with earlier-stage symptoms, which indicates a common relationship between symptom expression and fruit maturity. Once the fruits are mature, spot development apparently ceases.

The true cause is as yet unknown. All attempts to isolate microörganisms (bacteria and fungi) from the spot lesions have been negative, suggesting that the causal agent may be in the nature of a physiogen or virus.—E. M. HILDEBRAND, Cornell University, Ithaca, N. Y.

*Additional Records of Violet Scab.*—The geographic range of scab of violet (*Viola*) caused by *Sphaceloma violae* Jenkins,[1] includes the United States, new South Wales, and the Union of South Africa. Only the original record[2] of the disease in the Union of South Africa is at hand. There are

[1] Massey, L. M., and A. E. Jenkins. Violet scab caused by *Sphaceloma*. Cornell Univ., N. Y. Agr. Exp. Stat. Mem. 176. 9 pp. 1935.

[2] Jenkins, Anna E. Additional records of violet scab. Pl. Dis. Rptr. 22: 86–88. 1938.

several reports[1,3,5,6] from Australia, however, and in two instances[3,4] attention is called to its extreme destructivity to commercial violet culture. The following quotation is from the statement of 1941:

"Violet scab, which has been known to occur in this State since 1934, when it was discovered in a Richmond garden, is now common and widespread, in some seasons causing considerable loss to commercial growers. Last year, for example, in a metropolitan garden where a daily harvest of fifty dozen bunches was normally obtained, scab spoiled almost the complete crop."

In 1937 the disease had been found in 16 localities in New York, Pennsylvania, New Jersey, Maryland, Virginia, South Carolina, Georgia, Florida, and Alabama. Later it was reported[5] from the District of Columbia, on *Viola* sp., now identified as *Viola cucullata* Ait.

Violet scab occurs also in Massachusetts, Connecticut, North Carolina, Mississippi, and Texas, and in certain other localities in the States previously named. Among the new violet suscepts represented by the more recent records of the disease are *Viola jooi*, *V. priceana*, or *V. vilmoriniana*. The disease had previously been reported on *Viola cucullata*? but heretofore not on plants definitely identified as this species.[6] It was first observed on English violet (*V. odorata* L.), but until now it has not been recorded on the white-flowered variety. *V. cucullata* is a native of northeastern and *V. priceana* of southeastern United States; *V. jooi* is from southeastern Europe, and *V. vilmoriniana* from France. In transmitting the specimen of *V. jooi* Professor Whetzel wrote:

"This interesting and valuable species is one of the most beautiful in my garden. It flowers abundantly in spring before the leaves come out and again in autumn when cool weather comes on. It was recently discussed by R. T. Clausen in 'Gentes Herbarum' (4: 315. 1940)."—ANNA E. JENKINS, Bureau of Plant Industry, Washington, D. C.

*Physiologic Races in Urocystis tritici.*[1]—Physiologic specialization in *Urocystis tritici* Koern. apparently has not been extensively investigated. Verwoerd[2] obtained no evidence of physiologic races among several collections tested by him in South Africa, including a collection from the United States. On the other hand, Yu, *et al.*[3] described 5 races occurring in China.

[3] Anonymous. Violet Scab. Agr. Gaz. N. S. W. 52: 103. 1941.

[4] Noble, R. J. Australia: Notes on plant diseases recorded in New South Wales for the year ending 30th June, 1938. Internat. Jour. Pl. Protect. 13: 25M–26M. 1939.

[5] Jenkins, Anna E. Unusual collections of destructive fungi on plantain and violet in the District of Columbia. Pl. Dis. Rptr. 24: 370–372. 1940.

[6] Compare footnote 1, p. 7.

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, U. S. Department of Agriculture, and the Washington Agricultural Experiment Station.

[2] Verwoerd, Len. The biology, parasitism, and control of *Urocystis tritici* Koern., the causal organism of flag smut in wheat (*Triticum* spp.) and recording the occurrence of *Urocystis occulta* (Wallr.) Rab., in South Africa as the cause of "stem smut" in rye. Union of So. Africa Dept. Agr. Science Bull. 76, 59 pp. 1929.

[3] Yu, T. F., L. Hwang, and C. T. Tsiang. Varietal resistance and susceptibility of wheats to flag smut (*Urocystis tritici* Koern.). III. Physiologic specialization in *Urocystis tritici* Koern. Chinese Bot. Soc. Bull. 2: 111–113. 1936 [Rev. Appl. Mycol. 16: 305. 1936].

The identity of these was based on the results obtained from 4 years' tests on 10 wheat varieties. No other evidence of specialization in this fungus has been reported.

Although the occurrence of flag smut of wheat in the United States was first reported in 1919,[4] its distribution has been confined, until recently, to certain areas in Illinois, Kansas and Missouri. It was observed in 1940 near Goldendale, Washington,[5] and was apparently more widely present in 1941.[6] Investigations were begun to determine whether this smut in these two widely separated regions was caused by the same or different physiologic races.

Two collections of *Urocystis tritici* were used, one from Federation and certain other spring wheat varieties grown in the greenhouse at Arlington Farm, Virginia (originally from Leavenworth, Kans.), and the other a composite of spores from Hymar (C. I. 11605), Rex (C. I. 11689), and Hybrid 128 (C. I. 4512), winter wheat varieties, collected in 1941 near Goldendale, Wash. The inoculum was prepared by grinding smutted plant parts in a hand sausage mill and then separating the powdered spore material from the coarser plant parts with the aid of a soil sieve.

Seed of more than 100 varieties of winter and spring wheat was inoculated by shaking it and spores together in a glass vial or Petri dish. It was then planted in the greenhouse at Pullman, Wash. (1941 and 1942), Arlington Farm, Virginia (1941), and Beltsville, Maryland (1942). During the period of preemergence the temperature was maintained at approximately 15°–20° C. At Pullman, following complete emergence of the seedlings, Semesan solution was applied at intervals of 3 or 4 days for about 2 weeks in order to prevent excessive loss of plants from seedling blight.

Smut percentages were based on plant counts. At Pullman, the smutted plants were removed from the rows as the smut became evident. At Arlington Farm and Beltsville, the final counts were made when the normal plants had reached maturity. Both completely and partly smutted plants were included, and recorded separately, in the counts of smutted plants. Plants were considered smut-free if no infection was evident by heading time, although in certain varieties it was observed that smutted late tillers occasionally were produced by apparently smut-free plants. Varieties of this type, however, were not used for race differentiation.

These tests proved that the 2 collections of *Urocystis tritici* used represent 2 distinct physiologic races. This is clearly indicated by the reaction of the varieties listed in Table 1. The race from the Kansas smut is designated as race 1, and that from the Washington smut as race 2.

Certain varieties not listed in table 1 also exhibited differential reactions to these races; still others were resistant to both races. It is notable that differentiation of the two races is exhibited by resistance to race 1 and sus-

[4] Humphrey, H. B., and A. G. Johnson. Take-all and flag smut: Two diseases new to the United States. U. S. Dept. Agr. Farmers' Bull. 1063, 8 pp., 1919.

[5] Heald, F. D., and C. S. Holton. Flag smut of wheat found in Washington. Plant Dis. Rptr. 24: 382. 1940.

[6] Holton, C. S. Flag smut of wheat in Washington. Plant Dis. Rptr. 25: 335–336. 1941.

TABLE 1.—*Percentage of smutted plants in 6 varieties of wheat inoculated with 2 races of Urocystis tritici at Pullman, Wash., Arlington Farm, Va., and Beltsville, Md., 1941 and 1942*

| Variety | C.I. No. | Place; date tested | Plants from seed inoculated with | | | |
|---|---|---|---|---|---|---|
| | | | Race 1 | | Race 2 | |
| | | | Total | Smutted | Total | Smutted |
| | | | *Number* | *Per cent* | *Number* | *Per cent* |
| Federation | 4734 | Pullman, 1941 | 10 | 100 | 24 | 63 |
| | | " '41–'42 | 19 | 100 | 21 | 90 |
| | | " 1942 | 17 | 76 | 17 | 65 |
| | | Arlington, 1941 | 37 | 60 | 40 | 73 |
| | | Beltsville, 1942 | 50 | 46 | 48 | 69 |
| Oro × Federation-1 | 11914 | Pullman, '41–'42 | 17 | 82 | 21 | 86 |
| | | " 1942 | 17 | 82 | 18 | 83 |
| | | Beltsville, 1942 | 47 | 60 | 45 | 44 |
| Oro × Federation-38 | ..... | Pullman, 1941 | 35 | 0 | 34 | 79 |
| | | " '41–'42 | 22 | 0 | 21 | 86 |
| | | " 1942 | 22 | 0 | 13 | 69 |
| | | Beltsville, 1942 | 40 | 0 | 51 | 59 |
| Oro × Federation-40 | ..... | Pullman, 1941 | 17 | 0 | 31 | 78 |
| | | " '41–'42 | 23 | 0 | 22 | 73 |
| | | " 1942 | 22 | 0 | 21 | 29 |
| | | Beltsville, 1942 | 50 | 0 | 45 | 42 |
| Oro × Federation-26 | ..... | Pullman, 1941 | 16 | 25 | 34 | 21 |
| | | " '41–'42 | 17 | 65 | 19 | 100 |
| | | " 1942 | 24 | 29 | 18 | 83 |
| Baart | 1697 | Pullman, 1941 | 13 | 15 | 30 | 80 |
| | | " '41–'42 | 20 | 10 | 21 | 62 |
| | | " 1942 | 19 | 16 | 17 | 59 |
| | | Arlington, 1941 | 51 | 16 | 47 | 9 |
| | | Beltsville, 1942 | 48 | 4 | 44 | 36 |

ceptibility to race 2, whereas the reverse reaction has not been observed on any varieties tested.

It may be added, also, that while the reaction of Oro to the 2 races has not been tested under parallel conditions, it has been found susceptible to the Washington smut under greenhouse conditions and highly resistant to or immune from the Kansas smut, both in the greenhouse and in the field. Thus, apparently, the selections Oro × Federation-1 and Oro × Federation-26 are segregates that have the Federation type of susceptibility to both races, while Oro × Federation-38 and Oro × Federation-40 have the Oro type of reaction, *i.e.*, resistance to race 1 and susceptibility to race 2.—C. S. HOLTON and A. G. JOHNSON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

*Cultural Differences Among Single Basidiospore Isolates of Rhizoctonia solani.*—While many strains of *Rhizoctonia solani* Kühn differing in cultural characters and pathogenicity have been reported, little evidence exists as to their origin. Müller[1] has reported that some single basidiospore isolates from a basidial mat differed in cultural characters and pathogenicity.

[1] Müller, K. O. Untersuchungen zur Entwickelungsgeschichte und Biologie von *Hypochnus solani* P.u.D. (*Rhizoctonia solani* K.). Arb. Biol. Reichsanst. Land-u. Forstw. **13**: 197–262. 1924.

TABLE 1.—*Cultural strains secured from single basidiospore isolates of Rhizoctonia solani*

| Basidial mat | Host | No. cultures isolated | No. cultures compared in plates | No. cultural types |
|---|---|---|---|---|
| A | Lima bean | 7 | 6 | 6 |
| B | " " | 24 | 21 | 21 |
| C | " " | 52 | 22 | 22 |
| D | " " | 83 | 24 | 24 |
| E | Alligator weed | 60 | 13 | 13 |
| F | " " | 30 | 19 | 19 |
| G | Irish potato | 57 | 12 | 12 |
| H | " " | 4 | 4 | 4 |
| I | " " | 3 | 3 | 3 |
| J | " " | 75 | 29 | 29 |
| Total | | 395 | 153 | 153 |

Ryker and Exner[2] stated that some variation occurred in cultures from basidiospores found in nature.

An excellent opportunity developed in the summer of 1942 to make a more careful study of single basidiospore cultures. Basidial mats were very abundant on Lima bean, Irish potato, and alligator weed (*Alternanthera phylloxeroides*); and 395 single basidiospore isolates were obtained from 10 mats. Cultures from the same mat differed so strikingly it was felt the results were of sufficient value to report.

The cultures were first compared in tubes of potato-dextrose agar. The various types found among the isolates from each mat were then compared one or more times in triplicate Petri plates of potato-dextrose agar at 28° C. The rate of growth was determined from the 24th to the 48th hour.

In table 1 there have been brought together the data concerning the number of isolates secured from each mat, and the number of culturally distinct types obtained from each mat. It is noted that as many as 29 distinct cultural types were obtained from a basidial mat. These differed in the size, shape, number, and arrangement of the sclerotia, color of the mycelium, topography of the cultures, and rate of growth. Figure 1, A, shows

TABLE 2.—*Growth in millimeters, from the 24th to the 48th hour, of 21 cultures of Rhizoctonia solani from mat B on Lima bean (av. of two experiments)*

| Culture | Rate of growth | Culture | Rate of growth | Culture | Rate of growth | Culture | Rate of growth |
|---|---|---|---|---|---|---|---|
| B–1 | 31.3 | B– 6 | 33.2 | B–11 | 32.7 | B–17 | 28.2 |
| B–2 | 35.7 | B– 7 | 32.2 | B–12 | 31.5 | B–19 | 28.8 |
| B–3 | 33.0 | B– 8 | 32.2 | B–14 | 28.5 | B–20 | 36.2 |
| B–4 | 27.0 | B– 9 | 27.7 | B–15 | 36.0 | B–21 | 26.3 |
| B–5 | 35.7 | B–10 | 31.2 | B–16 | 28.8 | B–22 | 37.2 |
| | | | | | | B–23 | 31.8 |

Difference necessary for significance at 5% pt. = 2.0.
1% pt. = 3.8.

[2] Ryker, T. C., and Beatrice Exner. A comparative study of four species of Rhizoctonia. (Abstract) Phytopath. 32: 8. 1942.

6 cultures from the same mat, which differed in cultural characters, and figure 1, B, shows that duplicate plates of the same culture were very simi-

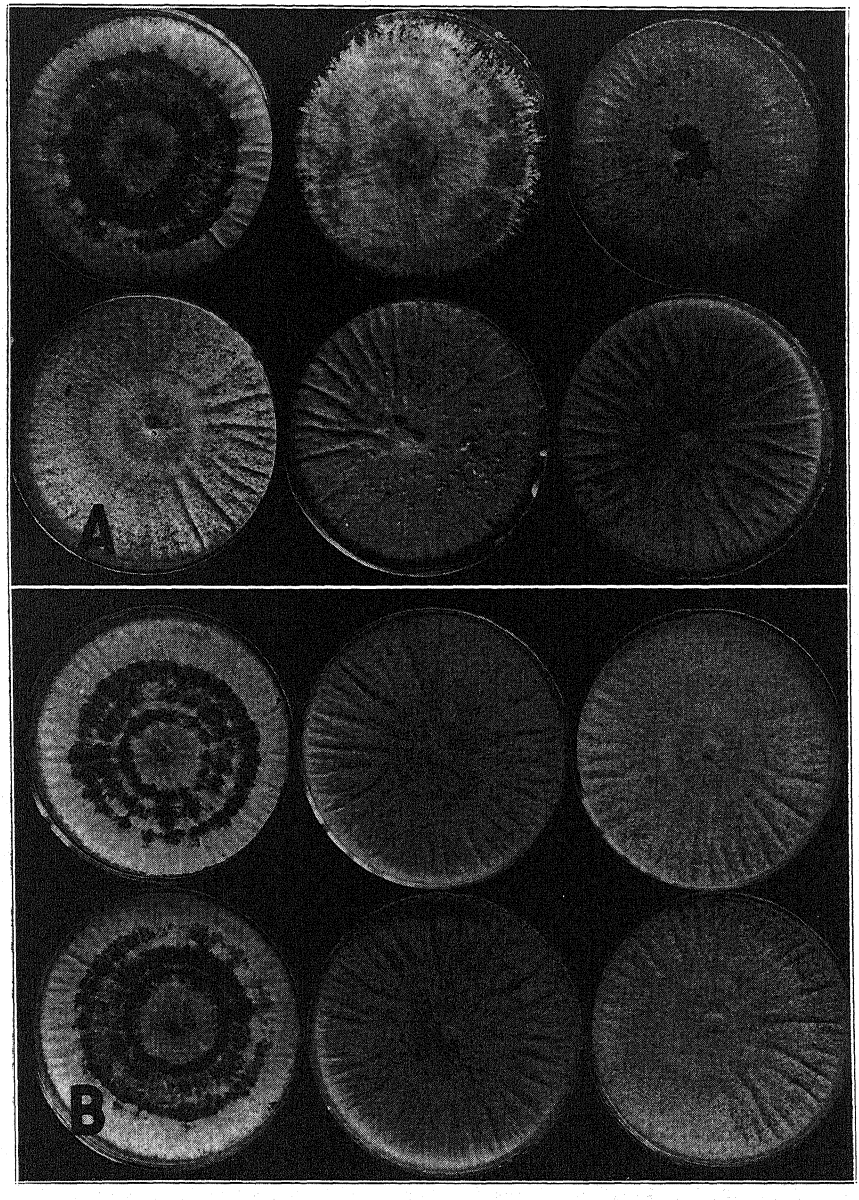



FIG. 1. A. Six cultural types of *Rhizoctonia solani* from mat D. B, Duplicate plates of 3 cultures of *Rhizoctonia solani* from mat D.

lar. Table 2, which gives the results of two experiments on the rate of growth of cultures secured from mat B, shows that the cultures differed

significantly in this respect. Although the data are not given, differences were found in growth rate among the isolates from the other 9 mats.

These results indicate that some type of segregation occurred in the formation of basidiospores and that different strains of *Rhizoctonia solani* arose as a result. Whether these cultures differ also in pathogenicity is being studied.—BEATRICE EXNER and S. J. P. CHILTON, Department of Plant Pathology, Louisiana Agricultural Experiment Station, Baton Rouge, La.

# INCREASE OF PATHOGENICITY IN TOMATO-WILT FUSARIUM

FREDERICK L. WELLMAN

(Accepted for publication June 26, 1942)

The tomato-growing industry has long experienced serious losses from the wilt caused by *Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R. Many workers are engaged in the development of wilt-resistant tomato varieties and it has become necessary to know more about changes in pathogenicity that may occur in the causal fungus.

The general practical and theoretical considerations of variability in pathogenic fungi have been discussed by many workers and summarized by Stakman (11), Brierley (2), and Das Gupta (4). The more immediate questions and implications of the effect of this variability on the tomato-wilt-resistance breeding program have been indicated by Boswell (1) and Haymaker (5). The studies reported here were designed to add to the essential understanding of the possible variations in virulence that must be considered in connection with testing wilt-resistant tomato stocks.

These studies are concerned with variability in the pathogenicity of the isolates employed in connection with disease resistance. Past experience has shown (15, 16) that under the usual laboratory handling, the virulent types of the organism commonly exhibit an unquestionable tendency to change, through saltant action, to mild types. However, it is well known that a relatively high virulence of the pathogen appears to be maintained over a long period of time in "wilt-sick" fields. This sustained virulence in nature had seemed to be somewhat in disagreement with the laboratory findings of a general tendency of variation towards milder pathogenicity, although there has been some suggestion (16) that the variation is not always "downward." This later investigation has added substantial evidence in support of variation towards higher virulence.

## MATERIALS AND METHODS

The purpose of this work was to determine, first, whether an increase of virulence of the wilt fungus occurred through variation in culture; second, the relative hardiness of mild and virulent types to conditions that may select out the latter, and third, comparative competitive capacities of virulent and mild types of the pathogen in culture and in the host.

The media employed were Tochinai's liquid medium (15) and standard potato-dextrose agar (15), and Wellman's differential medium (14). All Fusarium isolates were from the collection used in earlier studies (15, 16). The reselected and "pure-lined," mild type A15–8 and the virulent R5–6, used for comparative and reference purposes, have been studied and described elsewhere (14). The tomato varieties Bonny Best and Marglobe were the same as those employed in earlier work (12, 15, 16). All cultures were incubated at 28° C. Those aged were held at uncontrolled laboratory temperatures without attempting to reduce desiccation.

In spore-germination and single-spore-isolation studies, the suspensions were spread over hardened agar surfaces. Dilutions were such that around 5 to 20 spores occurred in a microscopic field under low power magnification. Around 30 microscopic fields were examined in marked areas in each Petri dish. Germination of conidia was studied after 18 to 32 hours of incubation and that of chlamydospores after 64 to 68 hours. Where spores appeared dead they were incubated 5 days before being discarded. Only well-isolated germinating spores were used for transfer, and these were picked from agar surfaces after growth was macroscopically visible.

Estimates were made of the number of spores included in a germination series as follows: 20 loopfuls of spore suspension from a loop 1 mm. in diameter, were placed on isolated spots on 2 per cent agar. Spores in the drops were then counted under the microscope and an average per loopful was thus obtained. An estimate of the number of spores was made from the number of loopfuls of suspension spread on agar surfaces to be incubated.

Five growth classes of the genus *Fusarium* have been defined elsewhere (14, 15, 16), but, for convenience, these are herewith briefly described as they appeared on Wellman's differential medium. Relative pathogenicities characteristic of the classes (Table 1) also are indicated.

TABLE 1.—*Pathogenicity values of the tomato wilt Fusarium based on disease effects on tomato seedlings at the 8-leaf stage, after inoculation and growth under standard conditions*

| Pathogenicity value[a] | Descriptive designation | Occurrence of darkened vascular bundles | | Collapse of stem |
|---|---|---|---|---|
| | | In hypocotyl | In epicotyl | |
| 0 | Healthy | None | None | None |
| 1 | Mild | In part of tap root | " | " |
| 2 | " | In all of root | " | " |
| 3 | " | Up to cotyledons | " | " |
| 4 | Medium | ..... | Above cotyledons | " |
| 5 | " | ..... | Above juvenile leaves | " |
| 6 | Severe | ..... | Above leaf 3 | " |
| 7 | " | ..... | Above leaf 4 | " |
| 8 | Very severe | ..... | Above leaf 5 | " |
| 9 | " | ..... | Above leaf 7 | " |
| 10 | Dying | ..... | In apical bud | Tip wilted |
| 11 | " | ..... | ..... | Tip drooping |
| 12 | " | ..... | ..... | Upper third collapsing |
| 13 | " | ..... | ..... | Upper half collapsing |
| 14 | " | ..... | ..... | Upper 4/5th collapsing |
| 15 | Dead | ..... | ..... | All collapsed |

[a] Compare with values defined in another report (12).

(R) Raised aerial mycelium, fine woolly white; slight reticulations over mat; undermat tender (easily cut), of light color; no appressed halo about colony. Pathogenicity around 15 to 13; most virulent of the "V" types.

(RS) Raised aerial mycelium with sclerotoid bodies; hyphae mostly fine with some coarse strands, white and fluffy; undermat tender, with dark

sectors; edges of colony often appressed. Pathogenicity 15 to 8; very irregular but considered a "V" type.

(IR) Intermediate raised; aerial mycelium somewhat coarse; narrow, partly appressed halo around colony; undermat light-vinaceous[1] shades, moderately tender. Pathogenicity 9 to 5; moderately virulent "V" type.

(IA) Intermediate appressed; scant, greyish, aerial mycelium, coarse, with wide halo of appressed growth; tough, lavender to tawny undermat. Pathogenicity 5 to 3. Moderately mild "M" type.

(A) Appressed, little or no aerial mycelium; slimy surface; dark vinaceous-purple to tawny, with tough mat. Pathogenicity 3 to 0. The mildest of the "M" types.

Bonny Best plants were employed in all pathogenicity tests, and disease values were obtained by the standard technique already described (12). In the present studies, however, values were recorded in all classs from 0 to 15 as indicated (Table 1).

In the plant-inoculation tests, cultures were held until, according to previous findings (14), mats had attained approximately their heaviest growth in the flasks of liquid medium. Cultures of V types were grown 9 days, those of the M types 22 days; when a culture was composed of the 2 types combined and in the same flask, it was held 15 days. Mats were removed from the liquid, drained a few minutes on filter paper, and the quantity of growth determined as the amount of water displaced by a mat. In preparing inoculum, a 10-cc. volume of fungus growth was added to each 190 cc. of water. When V and M isolates were grown separately and then mixed just before use as inoculum, a 5-cc. volume of each mat was included to make the final 200 cc. of suspension. In all cases the fungus material was thoroughly beaten 3 minutes in a Waring Blendor to insure thorough distribution of finely divided particles of the pathogen in the suspension.

## INCREASE OF VIRULENCE BY USE OF CULTURE METHODS

### Development of Virulent Saltants in Colonies of Mild Type

It has been repeatedly observed that the usual saltations in tomato wilt Fusarium cultures have been from the R type "down" towards A (15, 16), that is from comparatively high virulence (V) towards definite mildness (M) in pathogenicity. Many thousand mass cultures from 62 "parent" isolates were used, all of which were originally of monosporic origin (14, 15). All isolates of the raised or intermediate classes produced appressed sectors in culture.

In the present studies each variant that appeared in each one of the more than 3000 IA and A cultures examined was always compared with the isolate from which it came. The agar plate cultures each had from 1 to 9 distinctly visible sectors differing in appearance from the rest of the mycelium in the plate. In only 8 cases were the sector variants of a distinctly more raised, or virulent (V) type than the definitely appressed, or mild (M) culture from

[1] All color designations used in this paper are according to standards given in Ridgway (9).

which they came. Tests were made of these eight variants, run in parallel with the cultures of their origin and with known typical V and M types of the organism. It appeared (Table 2) that the differences in culture class

TABLE 2.—*Comparative virulence of 8 sector variants of the tomato wilt Fusarium found in two out of a total of about 3000 colonies[a] of mildly pathogenic type*

| Culture class | Isolate tested | Pathogenicity[b] value, mean and S.E. |
|---|---|---|
| A[c] | (Parent culture) | 1.70 ± 0.02 |
| IA | Sector a | 2.55 ± 0.02 |
| IA | Sector b | 3.15 ± 0.23 |
| IA | Sector c | 3.50 ± 0.47 |
| IR | Sector d | 5.40 ± 1.80 |
| IR | Sector e | 5.40 ± 0.55 |
| IR | Sector f | 6.45 ± 0.32 |
| IA | (Parent culture) | 3.73 ± 0.28 |
| IR | Sector g | 5.50 ± 0.58 |
| R | Sector h | 14.85 ± 0.01 |
| A | A15–8[d] | 1.00 ± 0.00 |
| R | R5–6[d] | 14.00 ± 0.34 |

[a] Each of these colonies except the two mentioned had from 1 to 9 distinct sectors of dark and appressed types differing in appearance from the rest of the Mycelium.
[b] Result of evaluations (cf. table 1) on 20 Bonny Best tomato plants.
[c] For description of culture-class designations see "Materials and Methods" above.
[d] Standard type (14) included for reference purposes.

of the variants that were more highly raised than their "parents" were definitely higher in pathogenicity.

The difference both in appearance and virulence between the light-colored IR sector f and the dark colored, slimy A colony whence it came was significant. The numerical pathogenicity from a test of the dark colored, slimy A "parent" colony was 1.70, but the fluffy IR sector f had a pathogenicity of 6.45. Another especially significant case was that of sector h, which was fully raised with a white woolly growth, but from a dark slate-purple mat of IA class with coarse, scanty mycelium over its surface. This h sector was highly virulent, with a pathogenicity value of 14.85, whereas its "parent" was mild in virulence with a value of 3.73.

## Isolates from Survivals of Thermal-Death-Point Tests

Thermal-death-point tests[2] were first instituted to see if there were differences in this respect between the mild (A15–8) and virulent (R5–6) isolates. Four tests (Table 3) were made and monoconidial isolates from survivors of the treatments were secured for observations as to possible growth variation in culture. The death point for conidia of the virulent culture was approximately 60° C., and that of the mild culture, a little above 50° C. Chlamydospores from either culture survived ten minutes' exposure to 60° C. This was probably close to the highest temperature they

[2] Made according to approved technique. Spore dilutions were from month-old potato-dextrose-agar cultures. Dilutions were in thin-walled test tubes, treated for 10-minute periods in a constant-temperature bath equipped with a standardized thermometer.

TABLE 3.—*Combined results of 4 thermal-death-point studies of conidial suspensions of virulent (R5–6) and mild (A15–8) isolates of tomato wilt Fusarium from which monoconidial isolations of survivors were secured to study for increased virulence*

| Temperature treatment[a] | Germination of conidia[b] | | Number of monoconidial isolates made[c] | | Chlamydospore germination | |
|---|---|---|---|---|---|---|
| | Virulent | Mild | Virulent | Mild | Virulent | Mild |
| Control[d] | 2822 | 2026 | 32 | 232 | 798 | 342 |
| 45° | 2841 | 1216 | 0[e] | 0[e] | ..... | ..... |
| 50° | 2245 | 504 | 0[e] | 281 | ..... | ..... |
| 55° | 232 | 0 | 206 | ..... | 187 | 199 |
| 60° | 4 | 0 | 4 | ..... | 102 | 13 |
| 65° | 0 | 0 | ..... | ..... | 0 | 0 |
| 70° | 0 | 0 | ..... | ..... | 0 | 0 |
| 75° | 0 | 0 | ..... | ..... | 0 | 0 |

[a] Treated for 10 minutes, all temperatures °C.
[b] Average number of survivors out of approximately 3000 conidia per test.
[c] On culturing no significant variations were observed in appearance from that characteristic of the culture of the isolate of their origin.
[d] At room temperature, about 24° C.
[e] No isolates made, since this treatment caused comparatively little destruction of conidia.

could withstand, since, when thus treated, their germination was greatly reduced. The largest percentage of chlamydospore survival after heating at 60° C. was recorded for the most virulent culture. None of the rather limited number of monoconidial isolates from survivors of these tests showed any significant deviation in culture appearance. It is, therefore, probable that there was no significant deviation in virulence among these.

## Isolates in Aged Cultures

Isolates that varied considerably in appearance and relative virulence were studied for differences in ability to withstand aging. Test-tube cultures on standard potato-dextrose-agar slants were stored in the laboratory for 1 to 40 months. From conidial suspensions the number of spores were plated out to determine and estimate their viability according to a method described under "Materials and Methods." Germination counts were obtained after incubating the spores at 18-, 24-, and 32-hour intervals. Monosporic isolates were made from all tests where germination occurred, and, whenever only a few spores appeared alive from large numbers plated out, every germinating spore was isolated that could be assuredly judged a single individual. These were grown on differential agar and in liquid medium. They were then compared with parent cultures for growth class (Table 4). The variants most obviously different in appearance were tested for relative pathogenicities, in comparison with the parents and other known cultures (Table 5).

It appeared (Table 4) that spores of both mild and virulent types were killed when aged 3 or $3\frac{1}{3}$ years. Laboratory storage for $2\frac{1}{3}$ to $2\frac{1}{2}$ years reduced the viability of both virulent and mild types, but was far more destructive of the mild type. Even $1\frac{1}{2}$ years of storage considerably in-

hibited the mild types, while 2 years of storage reduced germinability of the virulent types, but by a comparatively small amount. A single month of standing in the laboratory seemed to have no appreciable detrimental effect on spore germinability of either virulent or mild types.

Without including the data from any raised culture or results from the 1-month and 3- and 3½-year-old cultures, about 2400 spores were plated out from the surfaces of long-aged cultures of isolates "low" in culture class and mild in pathogenicity (Table 4). Of these spores only 489 germinated,

TABLE 4.—*Comparative germination of conidia from mild and virulent isolates of the tomato wilt Fusarium aged 1 to 40 months, together with culture types obtained from growth of samples of surviving spores*

| Isolate type and No.[a] | Months aged[b] | Conidia | | Distribution of monosporic isolates of different classes[d] | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Incubated[c] | Germinated | R | RS | IR | IA | A |
| V, 5R[d] | 1 | 2500 | 2396 | 180 | 0 | 0 | 0 | 0 |
| M, 15A | 1 | 2800 | 2469 | 0 | 0 | 0 | 0 | 380 |
| M, 10IA | 18 | 1000 | 110 | 36 | 11 | 11 | 18 | 4 |
| M, 3IA | 18 | 1000 | 61 | 6 | 1 | 1 | 51 | 2 |
| M, 1A | 18 | 1000 | 75 | 0 | 0 | 0 | 15 | 43 |
| M, 15A | 18 | 1000 | 103 | 0 | 0 | 0 | 1 | 59 |
| V, 5R | 24 | 1800 | 1593 | 67 | 0 | 0 | 0 | 0 |
| M, 15A | 24 | 2000 | 80 | 0 | 0 | 0 | 0 | 70 |
| V, 5R | 28 | 2100 | 1768 | 80 | 0 | 0 | 0 | 0 |
| M, 15A | 28 | 2200 | 19 | 0 | 0 | 3 | 10 | 6 |
| V, 5R | 30 | 3900 | 188 | 188 | 0 | 0 | 0 | 0 |
| M, 10IA | 30 | 3700 | 3 | 2 | 0 | 1 | 0 | 0 |
| M, 3IA | 30 | 2600 | 0 | 0 | 0 | 0 | 0 | 0 |
| M, 1A | 30 | 7100 | 38 | 0 | 0 | 1 | 10 | 27 |
| M, 15A | 30 | 4700 | 0 | 0 | 0 | 0 | 0 | 0 |
| V, 5R | 36 | 1500 | 0 | 0 | 0 | 0 | 0 | 0 |
| M, 15A | 36 | 1500 | 0 | 0 | 0 | 0 | 0 | 0 |
| V, 5R | 40 | 4200 | 0 | 0 | 0 | 0 | 0 | 0 |
| M, 15A | 40 | 4000 | 0 | 0 | 0 | 0 | 0 | 0 |

[a] V = virulent, M = mild. The isolates were the same as those used in previous studies under the same designations (14, 15 and 16).

[b] Aged at room temperature without guarding against desiccation.

[c] Numbers estimated.

[d] For definition of classes as indicated by letters, see above in this paper under "Materials and Methods."

377 of which were transferred as unquestioned monosporic isolates and studied in comparison with the growth of hyphae from week-old parent and type cultures of the 5 growth classes. Of these monosporic isolates, 91 appeared "higher" in culture class than the original culture from which they came.

Tables 4 and 5 show that isolates of low, intermediate, and high types came from single spores from cultures that appeared fully appressed throughout years of growth on artificial media. In turn intermediate cultures were studied and from them isolates were obtained that appeared completely free from any appressed or intermediate characters. Growth types also occurred among single spore isolates from intermediate cultures in which the isolates were of the two very highest classes.

Pathogenicity tests were made with some of the isolates that differed most

significantly from the type of their parent cultures. All these tests (Table 5), which were made simultaneously under identical conditions, substantiate the observation (Table 4) on culture class variation in monosporic isolates originating from appressed cultures. The parent isolates in these studies were quite similar to the mild type A15–8, which is low in pathogenicity and appressed in growth character. It is apparent that from such a seemingly

TABLE 5.—*Comparative pathogenicities of cultures of the tomato wilt Fusarium from representative*[a] *monosporic isolates of spores that survived after aging (Compare with table 4)*

| Parental culture[b] | | Age of culture in months | Monosporic isolate | | Pathogenicity[c] mean and S.E. |
|---|---|---|---|---|---|
| No. and class | Pathogenic type | | Growth class | Pathogenic type | |
| 5R | V | 30 | R | V | 14.00 ± 0.00[d] |
| | | 30 | R | V | 14.60 ± 0.30[d] |
| 15A | M | 28 | A | M | 1.00 ± 0.00 |
| | | 28 | IA | M | 2.13 ± 0.01 |
| | | 28 | IR | M to V | 3.60 ± 0.02 |
| 10IA | M | 30 | IR | V | 4.93 ± 0.31 |
| | | 18 | IR | V | 6.67 ± 0.53 |
| | | 30 | R | V | 8.73 ± 0.02 |
| | | 30 | R | V | 9.20 ± 0.37 |
| | | 18 | R | V | 11.60 ± 0.74 |
| | | 18 | R | V | 14.45 ± 0.45[d] |
| 3IA | M | 18 | R | V | 8.87 ± 0.54 |
| | | 18 | R | V | 11.20 ± 1.20 |
| | | 18 | R | V | 12.00 ± 0.68 |
| 5R | V | Not aged[e] | R | V | 14.00 ± 0.34 |
| 15A | M | do[e] | A | M | 1.33 ± 0.01[d] |
| 10IA | M | do[e] | IA | M | 2.93 ± 0.28 |
| 3IA | M | do[e] | IA | M | 3.73 ± 0.28 |
| R5–6 | V | do[e] | R | V | 14.85 ± 0.01[d] |
| A15–8 | M | do[e] | A | M | 1.20 ± 0.02 |

[a] A total of 82 isolates were tested for pathogenicities in a number of series. Those included in this table were all from one series of tests, and were selected to illustrate variation in virulence of monosporic isolates from cultures originally of mild type. Isolates from 5R included for comparative purposes.

[b] See foot note "a" in table 4.

[c] Pathogenicities all determined at the same time, tested on 15 Bonny Best plants except those marked "d."

[d] Data from test on 20 plants.

[e] Included for reference purposes.

weak and so-called "degenerate" type, variation proceeded "upwards" through all the intermediate steps to the highest type of culture class and pathogenicity; this last being comparable to the fully raised and highly pathogenic pure-line selection R5–6.

It is believed that these findings are of special significance. The whole gamut of pathogenicity was represented in these monosporic isolates from old cultures that had been originally of very mild form. Culturally, they ranged from appressed through intermediate forms to fully raised, and, pathogenically, from mild through intermediate to high virulence.

COMPETITIVE GROWTH OF MILD AND VIRULENT ISOLATES

## In Agar Cultures

The agar employed was of the differential formula previously devised (14) to exaggerate dissimilar growth characters of A15–8, a typical M isolate, and R5–6, a typical V isolate. The pieces for inoculum all came from just inside the edges of 3-week-old fungus colonies on agar. The pieces were cut in round disks of 3 sizes measuring 5.5 mm. in diameter (large); 3.0 mm. (medium); and 1.0 mm. (small). These disks were always placed with spore-bearing surfaces down on the agar. Combinations of inoculum were arranged in 13 different ways and grown at 28° C. in triplicate, and the test on each arrangement series was run 3 times.

The inoculum arrangements and results from their growth were as follows:

1. Large M disk alone; all new growth was M.
2. Large V disk alone; all new growth was V.
3. Large disks, M on top of V; all new growth was V.
4. Large disks, V on top of M; all new growth was M.
5. Large disks, edge of M placed 25 mm. from V; colonies met about 11 mm. from edge of V.
6. Large disks, M and V placed edge to edge; somewhat more of agar covered with M.
7. Large V disk, with medium M disk on top; all new growth was V.
8. Medium M disk, with large V disk on top; all new growth was V.
9. Large M disk, with medium V disk on top; all new growth was M.
10. Medium V disk, with large M disk on top; all new growth was M.
11. Large M disk with 2 small V disks placed on opposite sides along a diameter; growth mostly M with a comparatively narrow band of V extending in the line of the diameter.
12. Large V disk with 2 small M disks placed on opposite sides along a diameter; growth mostly M with comparatively narrow band of V extending perpendicularly to the diameter.
13. Matched halves of large M and V disks chopped and macerated together for inoculum; growth irregular, predominantly M type, scanty aerial hyphae, center with considerable V growth.

Growth effects from arrangements 5, 6, 11, 12, 13 are illustrated in figure 1.

These studies showed that the M and V isolates were close together in their relative capacities to grow on agar, but the M type was somewhat more aggressive and was able to outgrow the V. This was most strikingly exemplified in arrangements 11 (Fig. 1, D), 12 (Fig. 1, B. C), and 13 (Fig. 1, A), while it is shown to a lesser extent in 5 (Fig. 1, E) and 6 (Fig. 1, F).

## In Liquid Cultures

I have already reported (14) irregularities with regard to pH relations in cultures that were composed of V and M isolates growing in combination in

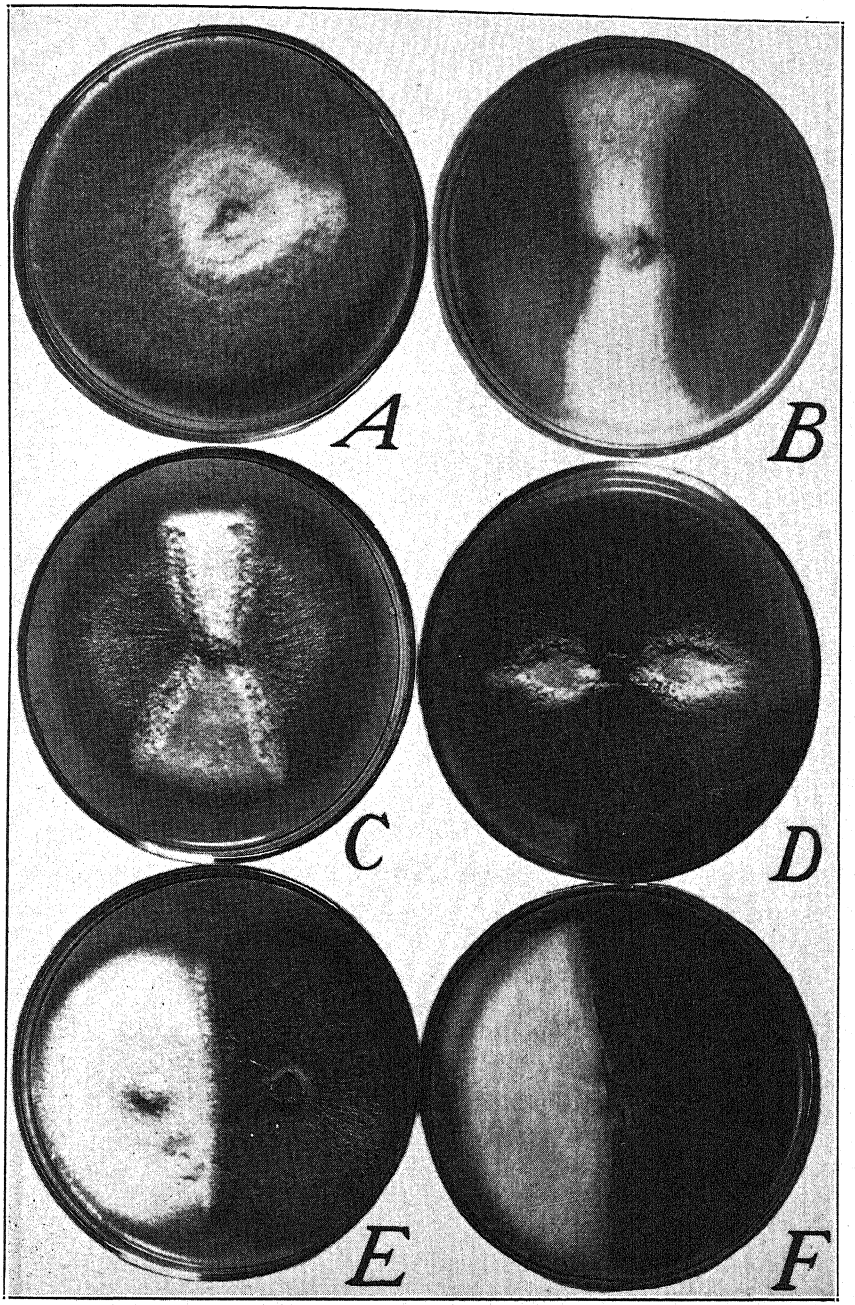



FIG. 1. Growth on agar of the white woolly virulent (V) and the dark slimy mild (M) isolates of *Fusarium bulbigenum* var. *lycopersici*. Inoculum arranged to test competitive growth of the two types. *A*. Matched halves of large, 5.5-mm. disks of V and M chopped and macerated together, planted in center. *B* and *C*. Large disk of V planted with small, 1-mm. disks of M on either side at ends of the transverse diameter. *D*. Large disk of M with small disks of V on either side at ends of the transverse diameter. *E*. Large disks of V and M planted 26 mm. apart. *F*. Large disks of V and M side by side. Note general predominance of mild growth in all arrangements, especially in *A*, *B*, *C*, and *D*.

flasks of liquids. The question of apparent competitive action arose in connection with such studies, and it seemed important to determine in more detail what might result from growth, in liquid, of virulent combined with mild types of the organism. A collection of V and M isolates was studied for changes in pH of the liquids (using techniques already described (14)), and also for appearance of growth on agar after a 14-day incubation period. The results of these studies are to be compared with but are not included in the data presented in table 6. Figure 2 shows the appearance of mats from cultures in a liquid medium of V and M types grown separately as pure cultures and also in combination. It is evident that the V character of growth was the more dominant.

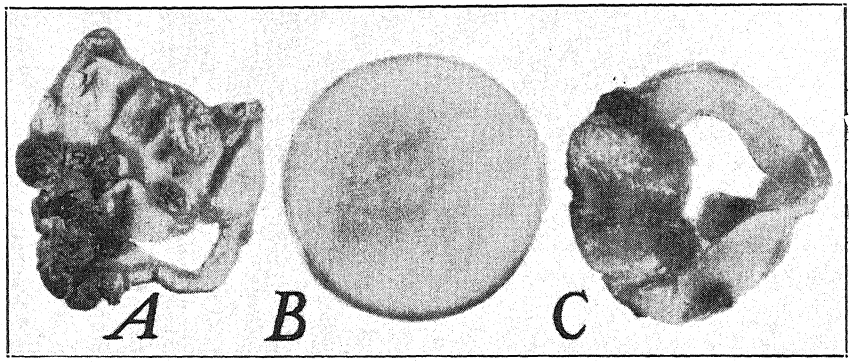



FIG. 2. Under surface of mats of *Fusarium bulbigenum* var. *lycopersici* grown in 100 cc. of Tochinai liquid in flasks. *A*. Pure growth of the virulent type of the organism. *B*. Pure growth of the mild type of the organism. *C*. Combined growth on using inoculum consisting of equal-size disks of both virulent and mild cultures. In liquid cultures the appearance of the combined growth is much nearer to that of the virulent type than to that of the mild.

For 17 pure M isolates all growth on agar was of the typical slimy appressed sort, light tawny to darker, with a few indistinct saltant areas, and a pH range in liquids of from 4.5 to 6.1 with a mean of 5.2. For 26 pure V isolates growth was of the white, woolly, raised sort, practically free from sectors, with a pH range of 7.7 to 8.4 and a mean of 8.1. For the 43 cultures obtained when mixed inoculum from the V and M isolates just described was used in combined cultures in flasks, the growth was variable in character but predominantly of the raised, white, woolly type with many slimy parts of tawny to darker color. In these liquids pH readings ranged from 5.6 to 8.3, with a mean of 7.7. In these studies of combined growth in liquid, the V type of growth appeared predominant.

In other studies of the same sort (Table 6) certain comparative determinations of the mat weights were made, and the competitive action of the V and M types of the *Fusarium* was studied in relation to pathogenicity. These experiments were carried on during two winters. Each experiment is a separate test and the results, therefore, are strictly comparable only within experiments. The data are, however, from experiments that were closely alike as regards the controllable environmental conditions.

TABLE 6.—*Comparative culture character and pathogenicity of virulent (V) and mild (M) isolates of tomato wilt Fusarium when grown in liquid cultures, with pure, combined, and mixed cultures used as inoculum*

| Experiment | Isolates | Data[a] on liquid cultures | | | | Pathogenicity[d] | |
|---|---|---|---|---|---|---|---|
| | | Character[b] | Class[c] | Liquid | Mat weight | Mean and S.E. | C.V. |
| | | | | *pH* | *mg.* | | |
| 1 | V–1 | Pure | R | 8.4 | 814 | 13.17 ± 0.31 | 2.35 |
| | M–1 | " | A | 5.8 | 382 | 0.83 ± 0.01 | 1.20 |
| | M–2 | " | IA | 5.9 | 407 | 4.75 ± 0.60 | 12.63 |
| | V–1 + M–1 | Combined | R & IR | 8.1 | 593 | 10.58 ± 0.41 | 3.88 |
| | V–1 + M–1 | " | IR | 8.0 | 512 | 12.50 ± 0.94 | 7.52 |
| | V–1 + M–1 | " | R | 8.3 | 600 | 13.00 ± 1.06 | 8.15 |
| | V–1 + M–2 | " | R | 8.0 | 666 | 8.42 ± 0.40 | 4.75 |
| | M–2 + M–1 | " | A | 5.6 | 412 | 1.00 ± 0.12 | 12.00 |
| 2 | V–2 | Pure | R | ..... | ..... | 14.85 ± 0.01 | 0.07 |
| | M–3 | " | A | ..... | ..... | 2.60 ± 0.01 | 0.38 |
| | V–2 + M–3 | Mixed | ..... | ..... | ..... | 6.40 ± 0.64 | 1.00 |
| 3 | V–3 | Pure | R | ..... | ..... | 14.85 ± 0.01 | 0.07 |
| | M–4 | " | IA | ..... | ..... | 2.30 ± 0.02 | 0.87 |
| | V–3 + M–4 | Mixed | ..... | ..... | ..... | 6.30 ± 0.57 | 9.05 |
| 4 | V–4 | Pure | IR | 8.0 | ..... | 8.80 ± 0.56 | 6.36 |
| | M–5 | Combined | A | 6.2 | ..... | 1.85 ± 0.01 | 0.54 |
| | V–4 + M–5 | " | IR | 7.2 | ..... | 8.85 ± 0.47 | 5.31 |
| | V–4 + M–5 | Mixed | ..... | ..... | ..... | 3.85 ± 0.63 | 16.36 |
| 5 | V–5 | Pure | R | 8.0 | 777 | 13.00 ± 0.33 | 2.54 |
| | M–6 | " | A | 5.8 | 472 | 1.35 ± 0.01 | 0.74 |
| | V–5 + M–6 | Combined | R | 7.6 | 707 | 12.07 ± 0.42 | 3.31 |
| | V–5 + M–6 | Mixed | ..... | ..... | ..... | 7.10 ± 0.70 | 9.86 |
| 6 | V–6 | Pure | R | 7.9 | 784 | 14.10 ± 0.58 | 4.11 |
| | M–7 | " | IA | 4.9 | 464 | 0.30 ± 0.01 | 3.33 |
| | V–6 + M–7 | Combined | IR & R | 7.7 | 767 | 13.40 ± 0.66 | 4.93 |
| | V–6 + M–7 | Mixed | ..... | ..... | ..... | 7.30 ± 1.63 | 22.33 |
| 7 | V–7 | Pure | RS | ..... | ..... | 11.40 ± 0.78 | 6.84 |
| | M–8 | " | IA | ..... | ..... | 1.50 ± 0.33 | 22.00 |
| | V–7 + M–8 | Mixed | ..... | ..... | ..... | 9.30 ± 1.05 | 11.29 |
| 8 | V–8 | Pure | R | ..... | ..... | 11.42 ± 0.74 | 6.48 |
| | V–9 | " | RS | ..... | ..... | 11.25 ± 0.81 | 7.20 |
| | V–8 + V–9 | Mixed | ..... | ..... | ..... | 11.00 ± 0.87 | 7.91 |
| 9 | V–10 | Pure | RS | ..... | ..... | 14.45 ± 0.45 | 3.11 |
| | V–11 | " | IR | ..... | ..... | 10.10 ± 0.84 | 8.32 |
| | M–9 | " | IA | ..... | ..... | 5.60 ± 1.53 | 27.32 |
| | M–10 | " | A | ..... | ..... | 0.30 ± 0.01 | 3.33 |
| | V–10 + V–11 | Mixed | ..... | ..... | ..... | 13.80 ± 0.73 | 5.29 |
| | M–9 + M–10 | " | ..... | ..... | ..... | 2.20 ± 0.51 | 23.18 |
| 10 | M–11 | Pure | IA | ..... | ..... | 4.75 ± 0.63 | 12.63 |
| | M–12 | " | A | ..... | ..... | 0.83 ± 0.01 | 1.20 |
| | M–11 + M–12 | Mixed | ..... | ..... | ..... | 1.00 ± 0.12 | 12.00 |

[a] Observations in triplicate.

[b] Using standard techniques (14) and incubated 12 days; pure = pure culture of isolate; combined = culture planted with equal bits of different isolates; mixed = equal portions of isolates that had grown as pure cultures were then mixed and thus used simultaneously as inoculum.

[c] See footnote "d," table 4.

[d] Each value represents a test on 20 plants of Bonny Best variety.

S.E. = standard error; C.V. = coefficient of variation.

Only a few mat weights were obtained, but it appears (Table 6) that when isolates were combined and grown in the same flask, they produced a weight, pH reaction. cultural appearance, and pathogenicity closer to that characteristic of the virulent than of the mild type. Virulent isolates had an average pathogenicity of well over 12, and mild isolates averaged around 2. However, when cultures of these isolates were mixed together mechanically, the resultant inoculum had an average pathogenic value of about 8. In instances when these M and V types were combined in the same flask and the resulting mat used as inoculum, the pathogenicity was below but close to the same as that produced by the pure V culture of the combination.

Pathogenicity tests of V and M isolates (whether pure, combined, or mixed cultures) were made in order to study further which type of isolate was predominant. Bonny Best tomato plants were inoculated (12). Superficial observations were recorded on the comparable state of wilting in plants of these tests at the time the plants were pulled and dissected for disease evaluation. There seemed to be great differences in uniformity of disease severity. Plants infected by the most typical and stable V or M cultures showed a uniform level of wilting, whether severe or moderate, in all the plants. In tests with plants inoculated with mats that had grown from combined V and M cultures, the level of wilting also was fairly even. On the other hand, the irregularity in wilting was quite noticeable in series inoculated with mechanical mixtures of pure cultures of V and M.

The comparative irregularity just mentioned is clearly indicated by the coefficient of variation as given in data from these studies (Table 6, experiments 3, 4, 5, and 6). Certain pure V and M cultures produced data with a small coefficient of variation. When the same V and M cultures were put together and mechanically mixed, the resultant data gave large coefficients. However, if inoculum came from a culture made of isolates that were of combined growth, the pathogenicity data showed a small coefficient. The coefficient of variation of the data from such a combined culture was of about the same magnitude as that obtained from data characteristic of the individual members of the combination culture.

### COMPETITIVE GROWTH OF MILD AND VIRULENT ISOLATES IN TOMATO PLANTS

### In the Susceptible Variety Bonny Best

In earlier studies (12, 13, 15, 16) reisolations were made from over 2000 plants that had been inoculated with purified, known types of the Fusarium. It was found that in general reisolations the same type of culture as that used for inoculum was recovered from infected plants.

It was thought advisable in the later work to standardize the method of making isolations; hence, only certain portions of the plants were used. Bits of tissue about 2 mm. square were excised from the vascular regions. In choosing the particular vascular strands to be tested for presence of the Fusarium, darkened bundles indicative of infection were selected where they occurred. If none were discolored, those were used that were an extension of

diseased tissues in the plant part below the point where dissections were made, or a bundle was used that led to an epinastic leaf, it being known (13) that such deflected leaves indicate fusarium infection.

The mild type of Fusarium, A15, has been used in many of the writer's studies (14, 15, 16). In standard tests on Bonny Best tomato, it has been found that most plants, inoculated with A15, show no discolored vascular bundles extending above the cotyledonary node. In these studies a total of 684 plants were thus inoculated and only 16 had darkened vascular tracts above the cotyledons. Reisolations were made from each of these 16 plants. In none of the cultures thus obtained was there a significant difference from A15, either in culture class or pathogenicity. In parallel studies, reisolations were made from over 300 plants infected with the virulent type, R5. Plants thus inoculated soon became severely diseased. On this account reisolations were made from plants in the early and late stages of wilt. In no case did the growth character of these reisolations vary from those typical for R5.

Plants were inoculated with mechanical mixtures of cultures of virulent and mild isolates of the wilt pathogen to determine whether there was competition between the virulent and mild types when they invaded plants. Certain isolates were chosen of widely different pathogenicities (Table 6) that were relatively stable in culture, and had markedly different culture appearances. Pairs were combined for inoculum that would be as nearly unmistakable as possible if reisolated from infected tissues. For example, practically colorless appressed M types were mixed with raised V types having a deep pink undermat; or dark purplish appressed M types were mixed with raised V types having white undermats.

The predominance of growth type as it appeared in mixed reisolation cultures from infected tissues was judged by macroscopic examination of what grew out from the diseased host parts. The type of the *Fusarium* covering the largest proportion of the surface of the reisolation plate was considered predominant. During these studies, 80 reisolation plates were observed on which growth type appeared to be about equally M and V, and all of these, in the end, were recorded as predominantly M. These methods were used regardless of the fact that, as was shown above in this paper and has been previously noted (14), the M type tends to grow over agar more rapidly than the V type. It should be pointed out that this method of determining the predominance of growth type may have resulted in unfair advantage in favor of the M type.

The 5 "parts" of the plant out of which the tissue bits were taken for reisolation were as follows: (1) root, tissues from below the transition region and down into the tap root 2 or 3 cm. below the transition region; (2) cotyledonary node, tissues from slightly above, below, and at the attachment of the cotyledons; (3) base, the internodes and leaf traces at the nodes where the juvenile leaves are attached; (4) middle, the recently mature but tender portion comprising the second internode down from the top leaf and apical bud; (5) apex, just below the apical bud.

Results from these studies show (Table 7) that all reisolates from plants inoculated with the pure V cultures were pure V, and all reisolates from

TABLE 7.—*Relative predominance of growth types in Fusarium reisolates from parts of Bonny Best tomato plants infected with virulent (V) and mild (M) types of the fungus that had been grown separately and then mixed for use as inoculum*

| Isolates[a] used for inoculum | Diseased plants | | Predominance of growth types, expressed as M/V, in cultures from plant parts[b] | | | | |
|---|---|---|---|---|---|---|---|
| | Number dissected | Range of disease[c] | Root | Cotyledonary node | Base | Middle | Apex |
| V–R5 + M–A15[d] | 138 | 1 to 15 | 56/82[e] | 36/97 | 45/90 | 30/94 | 10/82 |
| V2 + M1 | 20 | 2 to 15 | 7/13 | 2/17 | 5/13 | 6/11 | 5/11 |
| V1 + M2 | 19 | 5 to 13 | 6/13 | 10/9 | 7/12 | 7/11 | 6/11 |
| V1 + M3 | 24 | 8 to 14 | 10/14 | 11/13 | 11/13 | 5/19 | 5/18 |
| V3 + M3 | 16 | 1 to 4 | 7/9 | 5/11 | 5/11 | 0/8 | 2/5 |
| V5 + M1 | 20 | 8 to 15 | 5/15 | 10/10 | 3/17 | 9/10 | 3/11 |
| V9 + M4 | 18 | 1 to 15 | 8/10 | 8/10 | 10/8 | 4/14 | 3/15 |
| V1 + M4 | 20 | 2 to 12 | 9/11 | 7/13 | 4/14 | 4/11 | 3/4 |
| V5 + M5 | 20 | 1 to 15 | 2/18 | 1/19 | 1/16 | 0/12 | 0/11 |
| V6 + M5 | 20 | 4 to 15 | 2/18 | 3/17 | 2/18 | 4/13 | 1/10 |
| V7 + M12 | 20 | 4 to 15 | 7/13 | 5/15 | 5/15 | 0/12 | 2/12 |
| V8 + M12 | 6 | 11 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| V9 + M8 | 10 | 7 to 15 | 0/10 | 0/10 | 0/10 | 0/9 | 0/7 |
| V8 + M10 | 12 | 4 to 13 | 1/11 | 0/12 | 1/11 | 1/7 | 0/5 |
| V10 + M5 | 14 | 1 to 13 | 0/14 | 0/11 | 0/12 | 1/7 | 0/7 |
| V10 + M4 | 20 | 1 to 15 | 14/6 | 16/0 | 14/2 | 7/2 | 2/2 |
| V–R5[d] | 101 | 8 to 15 | 0/101 | 0/101 | 0/100 | 0/78 | 0/73 |
| M–A15[d] | 89 | 1 to 4 | 89/0 | 14/0 | 6/0 | 3/0 | 0/0 |

[a] Isolates chosen from those listed in table 6 that were widely different in pathogenic effect. Average value for V types was about 13.2, for M types about 1.5 (see table 1 for disease index values).

[b] Detailed description of plant parts and technique of isolation given in the text.

[c] Expressed in range of disease index (cf. table 1) of the plants used for reisolation purposes. These plants were pulled at irregular intervals so as to reisolate from those that differed considerably with regard to severity of disease.

[d] Included for reference purposes; V–R5 and M–A15 are the author's described R5–6 and A15–8 respectively (14).

[e] Reisolates were cultured and classified according to predominance of growth-type of *Fusarium* emerging from the bits of vascular system dissected from diseased plant parts. Expressed as M/V: numerator = number of plant parts from which the predominantly mild (M) type of pathogen developed; denominator = those from which came the predominantly virulent (V) type.

pure M inoculated plants were recovered as pure M. Reisolations from those seedlings inoculated with a mixture of M and V types of the pathogen were made as follows: 397 plants were used for the reisolation purposes, from which attempts were made to secure isolations from a total of 1985 plant parts. Of these parts, 1745 produced cultures of the pathogen, 1264, or 72 per cent, of which were predominantly V; from cotyledonary nodes, 70 per cent were predominantly V; from base parts above the node, 70 per cent were predominantly V; from middle parts, farther up, 76 per cent were predominantly V; and from apex parts, directly below the terminal bud, 84 per cent were predominantly V. When the isolates from tissues from plants infected with mixed V and M cultures were further cultured, it was observed that 92 per cent of all such cultures consisted of a mixture of both

V and M. Since only 8 per cent of all the cultures from plants inoculated with mixed cultures were found to be pure V types, it appears that both M and V were commonly present in those severely diseased seedlings in even the uppermost parts of the plants. It is, therefore, not alone the presence of the fungus in host tissues but its action that causes serious damage.

Results with 30 plants of the above studies (Table 7) deserve detailed discussion, since they represent the repeated observation that growth of M types of the Fusarium extended much farther into the vascular elements of plants infected with both M and V types than when infections resulted from M types alone. Inoculated plants were grown in parallel series under controlled conditions, and tissues were dissected out for reisolation of the pathogen after 9 days.

The first 10 plants had been inoculated with the M type, A15–8, and all appeared healthy before pulling. The roots of all these plants contained the M type of the Fusarium; and in two plants this type was reisolated from tissues slightly below the cotyledonary node. The second 10 plants were inoculated with the V type, R5–6, and were dying. All reisolations from any part of all these plants were the V type. The third group of 10 plants were inoculated with a mixture of these M and V types of the organism. All the plants were diseased, two being mildly wilted. In one of these the M type predominated half way up the stem; in the other the V type predominated, although the M type was recovering along with the V type up to the farthest point of invasion. From two other plants, more severely diseased, the reisolations showed good growth of both V and M with V slightly predominant; but here, again, the M had grown with the V and was recovered from infected vasculars near the apexes of these diseased plants. In two more of the severely wilted plants from this group a combination of M and V types of culture were recovered from the basal half of the plants, although isolates from the apical tissues of one plant were of pure V type. The last 4 plants of this group were collapsing. In the reisolations from them, mixtures of M and V types of the organism came from all tissues, even from the apices.

### In the Resistant Variety Marglobe

Both Bonny Best and Marglobe seedlings were used in these studies (Table 8). The isolates for inoculum came from the same lot as those in table 7. Certain of these were reselected and were designated as a. The V types were chosen among those that were raised, white, and produced nothing but non-septate microspores as conidia, while all M cultures were appressed, dark, and bore typically multiseptate macrospores. Any reisolate diagnosed as pure V, listed in table 8, was proved for purity by microscopic examination. Finding of any septate spores was considered sufficient to class the reisolate as impure for V, no matter what was deduced from its macroscopic appearance. The methods used were the same as those employed before, except that inoculated Marglobe plants, and likewise, Bonny Best plants inoculated with M, were grown for longer periods so that a weak infecting

organism or infections held in check by resistant plants, had ample opportunity to develop to a point at least slightly above the cotyledonary node.

Examination of the data (Table 8) from plants inoculated with mixed M and V isolates showed that in reisolations from hypocotyls of Bonny Best plants, the cultures recovered were about equally divided between those predominantly V and those predominantly M. In reisolations from hypocotyls of Marglobe plants, nearly half were impure (for type) cultures of

TABLE 8.—*Comparison of reisolates on agar from Bonny Best and Marglobe tomatoes inoculated with a mixture of virulent (V) and mild (M) types of wilt Fusarium*

| Host plants | Isolates[a] | Reisolation from plant parts indicated | | | |
|---|---|---|---|---|---|
| | | Hypocotyl[b] | | Epicotyl[b] | |
| | | Pure cultures (type observed) M/V | Mixed cultures (predominant type) M/V | Pure cultures (type observed) M/V | Mixed cultures (predominant type) M/V |
| Bonny Best | VR5 + MA15 | 0/0 | 6/4 | 2/3 | 4/1 |
| | VR5 + M1–a | 0/0 | 8/2 | 0/1 | 7/2 |
| | VR5 + M3–a | 0/0 | 4/6 | 1/2 | 1/6 |
| | V7–a + M5–a | 0/2 | 2/6 | 1/2 | 3/4 |
| | VR5–a + M8–a | 0/0 | 8/2 | 0/1 | 0/9 |
| | V10–a + M10–a | 0/0 | 3/7 | 0/0 | 4/6 |
| | V1–a + M2–a | 0/0 | 6/4 | 0/0 | 4/6 |
| | V7–a + MA15 | 0/0 | 2/8 | 1/3 | 4/2 |
| Marglobe | VR5 + MA15 | 0/1 | 3/5 | 0/9 | 0/1 |
| | VR5 + M1–a | 1/3 | 2/4 | 0/10 | 0/0 |
| | VR5 + M3–a | 1/4 | 4/1 | 0/9 | 0/1 |
| | V7–a + M5–a | 0/5 | 0/5 | 0/10 | 0/0 |
| | VR5–a + M8–a | 0/0 | 3/7 | 0/6 | 0/4 |
| | V10–a + M10–a | 0/1 | 0/9 | 0/10 | 0/0 |
| | V1–a + M2–a | 1/4 | 2/3 | 0/10 | 0/0 |
| | V7–a + MA15 | 1/5 | 1/3 | 0/9 | 0/1 |
| Bonny Best | VR5[c] | 0/10 | 0/0 | 0/10 | 0/0 |
| | MA15[c] | 10/0 | 0/0 | 10/0 | 0/0 |
| Marglobe | VR5[c] | 0/10 | 0/0 | 0/10 | 0/0 |
| | MA15[c] | 10/0 | 0/0 | 10/0 | 0/0 |

[a] The same isolates as those used in table 7, except those marked a, indicate reselection.
[b] Hypocotyl = taproot, transition region, and point just below cotyledons.
Epicotyl = points just above cotyledonary node, and in base, middle, and apex of plant.
[c] Included for reference purposes.

predominantly V type, and about 30 per cent were apparently pure V. In diseased epicotyl tissues, from just above cotyledonary nodes and up into the apices of the seedlings, the reisolations from Bonny Best plants were about 15 per cent pure V. Practically all the rest were impure for type, with about equal division between those predominantly V and those predominantly M. In comparable reisolations from Marglobe plants, over 90 per cent of the cultures were pure V type, which indicated common supersedence of the M type by the V as it invaded the upper parts of the more resistant host.

## DISCUSSION

These studies indicate methods whereby Fusarium isolates of highly

pathogenic nature may be obtained for use in wilt-resistance or other experimental research.

Where relative differences in disease effects are to be considered, it has been found essential that only well-purified isolates of the highest pathogenicity be employed. In the past it often has seemed advisable to use many widely different types of cultures of a pathogen in testing for disease resistance. These cultures usually were grown separately and mixed together for inoculum in the belief that a disease resistance reading, that is, the relative pathogenic attack by such a mixture, would be closest to the effect obtained under natural field conditions. The writer has concluded, however, that such mixing often may complicate results, as shown by examples given in the present paper. Such end data may be found to be full of distracting irregularities. When a number of variants of the organism are to be used simultaneously for general host reaction, they should not be grown separately and mixed just before the inoculum is applied, but should be grown together in combined culture and then used as inoculum. It would appear that a considerable advantage might be gained in the tomato wilt-resistance program by use of controlled inoculum from a single carefully purified Fusarium isolate.

Selection of an isolate should be thoroughly considered in comparison with other members, since study of widely divergent types of *Fusarium bulbigenum* var. *lycopersici* show that their characters may extend beyond the description given by Wollenweber and Reinking (17). This also has been observed by other workers (10), and one purpose of the writer's studies has been a better understanding of the wide pathogenic and cultural variability of the pathogen.

Earlier research already has disclosed (14) certain differences in the physiology of mild and virulent isolates of the tomato-wilt Fusarium. In the present work it has seemed that the virulent type of the organism is in several respects the most hardy and the most vigorous. In comparative tests of the viability of cultures of the mild and virulent isolates aged for about 2 years, most of the conidia died, but more failed to germinate from the mild isolate than from the virulent. On agar, the mild organism grew more rapidly than did the virulent, but where there was intimate competitive action through mixed growth of the two forms in liquid media, the virulent soon dominated the mild. In comparisons of thermal death points of conidia from mild and virulent isolates, the latter withstood a distinctly higher degree of heat. When susceptible Bonny Best tomato plants were inoculated with mixtures of mild and virulent types of the organism, both forms grew well up into diseased plants, but the virulent became the more abundant. When more resistant Marglobe plants were thus inoculated both forms of the fungus infected the plants, but the invasive capacity of the virulent type was so much greater that it commonly superseded the mild by the time it had reached the upper parts of these plants. All these observations indicate the much greater competitive capacity of the virulent as compared with the mild type of the wilt organism.

Variation towards mildness or virulence did not tend in either "direction" permanently. While the real nature of such reversible modification remains obscure, it would not appear to be a true mutation but rather a matter of adaptive variation. The modifications were seemingly enhanced by selective action under cultural manipulations, or the environment encountered in the host. Such adaptive variation may occur in nature and at times may result in a predominance of the more pathogenic type of organism in the field. It appears that adaptation towards increasing virulence might follow prolonged culture of resistant tomato varieties in soils infested with variant parasites that originally may have been of rather mild type.

During the past several years there have been recurrent reports (3, 6, 7, 8) of localized instances of serious wilt damage in tomatoes. These have occurred in spite of the use of the available commercial wilt-resistant or tolerant varieties. It is recognized that considerable variations in environment as well as accidental mixtures in host strains, may contribute to such apparent wilt damages as are cited. However, it is believed the studies here reported suggest a further explanation of unexpected losses to growers. Small numbers of highly virulent types of the Fusarium may develop through variant action. These being most effective competitors, would grow more successfully than the mild types in fields planted with highly tolerant or resistant hosts. The virulent type of the organism would multiply successfully in these plants, whereas the mild would be more or less held in check. On decay of the diseased plants increasingly larger quantities of spores of the virulent type of pathogen would be left in the soil. Other natural means, such as desiccation and heat, also might tend to further reduce the numbers of the milder types of the organism. The quantity of highly virulent forms in the soil might thereby increase and eventually be the cause of unexpectedly severe attack on plants otherwise known as commonly resistant to wilt.

Indeed, it would appear that the future permanence of a tomato industry reasonably free from wilt losses may depend on the continuing effort of plant breeders. They may well have to improve further the resistance of the tomato strains beyond what is now available, testing their crop varieties by inoculation with the most virulent types of the organism obtainable.

## SUMMARY

By far the greatest proportion of saltations in agar cultures of the tomato-wilt Fusarium (*F. bulbigenum* var. *lycopersici*) showed a lower degree of virulence than the parent cultures. Occasional variation in pathogenicity did take place in the reverse direction, although less than one sector in a thousand from mild cultures was of a distinctly higher virulent type.

The thermal death point of conidia from a virulent form of the organism was approximately 60° C., whereas that for a mild form was a little more than 50° C. With cultures that were 2 to 2½ years old, more conidia germinated from virulent isolates than from mild. Cultures from

surviving conidia from old cultures of mild types of the organism were mostly of higher pathogenic type than those from conidia that had not been allowed to age.

On agar surfaces the mild type of the organism grew faster than the virulent, but, grown as a mixture in liquid media in the same flask, the virulent was commonly predominant over the mild.

Susceptible host plants, under the conditions imposed, were ordinarily invaded by the mild form of the organism only up to the cotyledonary node. However, when plants were simultaneously inoculated with both a mild and a virulent form, the mild type proceeded parallel with the virulent invader into the upper parts of the plant. In these plants, however, the virulent form developed more extensively than the mild. In resistant hosts the mild isolates were more confined to the basal portions of the plant, even when there was a mixed infection. Also the virulent form was commonly precedent over the mild as it extended farther up towards the plant apex.

Changes in virulence were concluded to be in the nature of adaptive variation rather than true mutation.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND.

LITERATURE CITED

1. BOSWELL, V. R. Improvement and genetics of tomatoes, peppers, and eggplants. U. S. Dept. Agr. Yearbook 1937: 176–206. 1937.
2. BRIERLEY, W. B. Variation in fungi and bacteria. Proc. Int. Congr. Plant Sci. 2: 1629–1654. 1929.
3. COOK, H. T. Vegetable diseases in Virginia. U. S. Dept. Agr. Pl. Dis. Rptr. 19: 243–244. 1935.
4. DAS GUPTA, S. N. Saltation in fungi. Lucknow Univ. Studies 5: 66 pp. Newul Kishore Press (Lucknow). 1936.
5. HAYMAKER, H. H. Pathogenicity of two strains of the tomato-wilt fungus, *Fusarium lycopersici* Sacc. Jour. Agr. Res. [U.S.] 36: 675–695. 1928.
6. HUMPHREY, H. B., and J. I. WOOD. Diseases of plants in the United States in 1933. U. S. Dept. Agr. Pl. Dis. Rptr. Suppl. 86: 1–92. 1935.
7. MILLER, J. H. Tomato disease notes for Georgia for 1936. U. S. Dept. Agr. Pl. Dis. Rptr. 20: 350–355. 1936.
8. NANCE, NELLIE W. Diseases of plants in the United States in 1939. U. S. Dept. Agr. Pl. Dis. Rptr. Suppl. 128: 210–378. 1941.
9. RIDGWAY, R. Color standards and color nomenclature. 43 pp., 53 col. plates (Washington). 1912.
10. SNYDER, W. C., and H. N. HANSEN. The species concept in *Fusarium*. Amer. Jour. Bot. 27: 64–67. 1940.
11. STAKMAN, E. C. The need for research on the genetics of pathogenic organisms. Amer. Assoc. Adv. Sci. Publ. 12: 9–17. 1940.
12. WELLMAN, F. L. A technique for studying host resistance and pathogenicity in tomato Fusarium wilt. Phytopath. 29: 945–956. 1939.
13. ———. Epinasty of tomato, one of the earliest symptoms of Fusarium wilt. Phytopath. 31: 281–283. 1941.
14. ———. Difference in pH relations of some pathogenically variable strains of tomato Fusarium. Phytopath. 32: 271–287. 1942.
15. ———, and DOROTHY J. BLAISDELL. Differences in growth characters and pathogenicity of Fusarium wilt isolations tested on three tomato varieties. U. S. Dept. Agr. Tech. Bull. 705, 28 pp. 1940.
16. ———, and ———. Pathogenic and cultural variation among single-spore isolates from strains of the tomato wilt *Fusarium*. Phytopath. 31: 103–120. 1941.
17. WOLLENWEBER, H. W., and O. A. REINKING. Die Fusarien, ihre Beschreibung, Schadwirkung und Bekämpfung. 355 pp. (Berlin). 1935.

# OCCURRENCE, IDENTIFICATION, AND SPECIES VALIDITY OF THE BARLEY LOOSE SMUTS, USTILAGO NUDA, U. NIGRA AND U. MEDIANS

V. F. TAPKE[1]

(Accepted for publication June 12, 1942)

## INTRODUCTION

It has long been accepted that loose smut of barley is caused by *Ustilago nuda* Rostr., a floral-infecting fungus, which produces a deep internal infection of the seed practically beyond control by any simple treatment. About 20 years ago it was found (35) that, not infrequently, high infection could be produced by inoculating the seed as well as the flowers, and also (21, 22) that seed treatment with certain easily-applied surface disinfectants often resulted in excellent control. The discovery (29) of the seedling-infecting *U. nigra* and (30) that loose smut caused by it could be completely controlled by applying surface disinfectants to the seed seemed to explain this discrepancy.

The discovery of *Ustilago nigra,* however, raised certain questions regarding the identity and species validity of *U. nuda, U. nigra* and a third fungus said to be responsible for loose smut of barley, *U. medians,* as described by Biedenkopf (4). Experiments, therefore, were undertaken to determine the number and prevalence of species causing loose smut of barley in the United States, and whether the different species could be recognized by a microscopic examination and simple spore germination tests.

## EARLIER RESEARCH ON CHLAMYDOSPORE GERMINATION OF BARLEY LOOSE-SMUT FUNGI

During the 50 years since *Ustilago nuda* was described, its spore-germination and other characters have been observed extensively in laboratories throughout the world (3, 6, 13, 19, 28, 33). All reports fully agree that it produces the loose type of smutted head and that its chlamydospores are echinulate and, on germinating, normally produce only branching filaments (Fig. 1, A). According to Hüttig (16), *U. nuda* may be induced to form sporidia by germinating the spores at freezing temperatures. Thren (34), however, found that its promycelial cells separated under these conditions but formed no sporidia. It would seem probable, therefore, that the type of spore germination of *U. nuda* is sufficiently fixed to serve a useful purpose in species identification.

*Ustilago nigra,* also, is an echinulate-spore fungus. The chlamydospores, however, normally germinate by producing a promycelium bearing typically 4 lateral sporidia (30) (Figs. 1, B and 2, A). Referring to the "third

[1] The writer is indebted to M. A. McCall and A. G. Johnson, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for helpful advice regarding nomenclature and procedure in the present study. The writer is also indebted to Edith K. Cash for preparing the Latin description of *Ustilago nigra.*

species of barley smut known as *Ustilago nigra* or *Ustilago medians,*" Allison (2) also reported that it consistently formed sporidia on potato-dextrose agar. Ruttle (25) described several intermediate barley smuts, similar to *U. nigra,* that produced sporidia. In studies of *U. nigra* on grasses, Fischer (10) germinated the chlamydospores on potato-dextrose

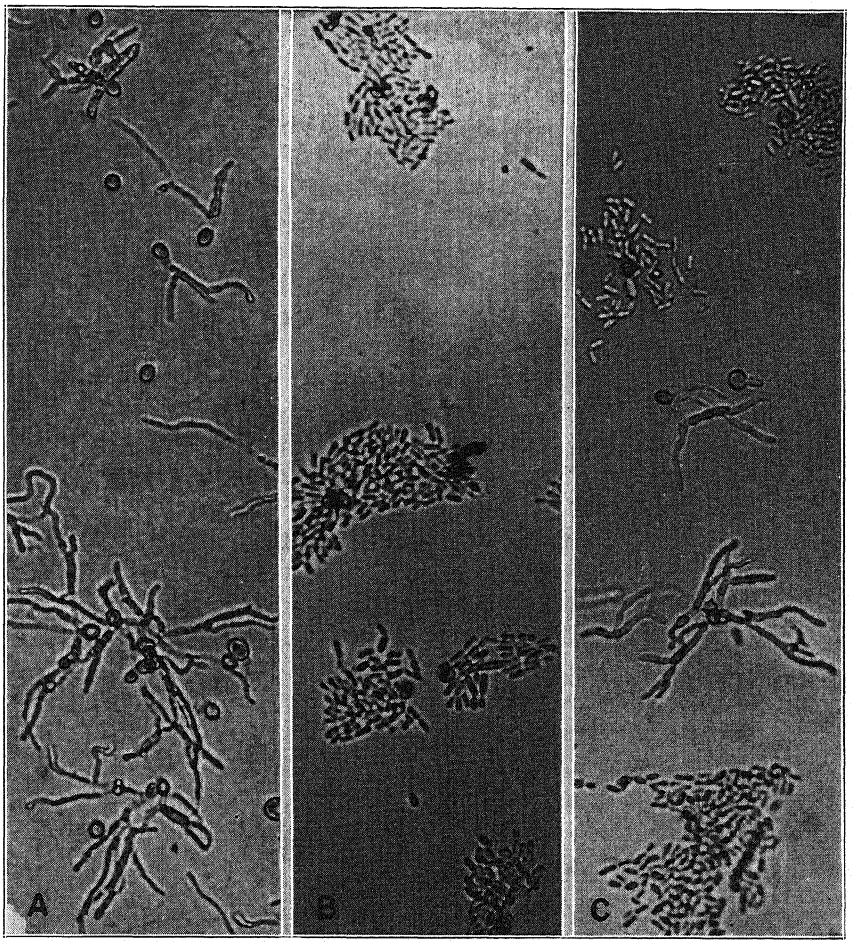



FIG. 1. The three types of spore germination obtained on potato-dextrose agar at 20° C., in the study of 500 barley loose-smut collections from different parts of the United States. A. The mycelial type of *Ustilago nuda.* B. The sporidial type of *U. nigra.* C. The mixed mycelial-sporidial type of *U. medians* that proved to be a mixture of *U. nuda* and *U. nigra.* ×275.

agar and obtained sporidia, as with *U. hordei.* This is good agreement and indicates that it should not be difficult to distinguish *U. nigra* from *U. nuda* through difference in type of spore germination.

*Ustilago medians* was described as forming echinulate chlamydospores that germinated in a most unique manner. When sown on gelatin, some produced the mycelial germination typical of *U. nuda;* but the majority

formed a promycelium and sporidia typical of *U. hordei* (Fig. 1, C). As noted by Biedenkopf (4): "Neben solchen Kolonien, die sich als von der nur Mycel bildenden *Ustilago Hordei* ansprechen liessen, fanden sich jedoch auch in grösserer Zahl solche, wie sie sich von Conidien bildenden Ustilagineen zu bilden pflegen."[2]

Suspecting that he might have in one smutted head a mixture of the echinulate-spore, mycelium-producing loose smut (*Ustilago nuda*) and the smooth-spore, sporidia-producing covered smut (*U. hordei*) ("ob in diesem Fall *Ustilago Jensenii* und *Ustilago Hordei* nicht etwa zusammen auf einer Ähre sich fänden"),[2] Biedenkopf examined the spores, but found none of covered smut in the loose-smut heads, because all of the spores were echinulate. In the second and final test the spores were sown in diluted plum decoction and germinated within 4 hours. Again, both the conidial and mycelial types of germination occurred. As noted by Biedenkopf: "Einige trieben einen meist dreizelligen Mycelschlauch und begannen nun in rascher Folge Conidien abzuschnüren, die sofort nach dem Abfallen in hefeartige Sprossung übergingen. Die meisten Sporen dagegen keimten zu Mycelien aus." In the first test, therefore, most of the spores formed sporidia but in the second test most of the spores formed mycelium.

Further observation of the cultures showed that the later developments of the mycelial and sporidial types of germination were identical with those normally characteristic of *Ustilago hordei* Bref.[3] and *U. jensenii* Rostr.[3]

Biedenkopf's excellent descriptions leave no doubt but that his *Ustilago medians* was an echinulate-spore loose smut of barley with a mixed mycelial-sporidial type of spore germination, similar to or identical with that produced by a mixture of *U. nuda* and *U. hordei.* Therefore, if *U. nuda, U. nigra* and *U. medians* have been correctly described, the morphological character that should distinguish the three species is the mycelial germination of the spores of *U. nuda,* the sporidial germination of those of *U. nigra* and the mixture of mycelial and sporidial types produced by the chlamydospores of *U. medians.*

## MATERIAL AND METHODS

The loose smut of barley here reported comprised 500 viable collections from the following States: Arkansas, Colorado, Delaware, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maryland, Michigan, Minnesota, Mississippi, Missouri, Montana, Nebraska, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Virginia, Washington, West Virginia, Wisconsin, and Wyoming.

Past research on the floral-infecting loose smut (*Ustilago nuda*) frequently has been hampered by its short-lived spores. This difficulty was met in the present study by storing the *U. nuda* and other collections at

[2] Biedenkopf applied the binomials *Ustilago hordei* Brefeld to the loose smut (*U. nuda* Rostr.) and *U. jensenii* Rostrup to the covered smut (*U. hordei* Lagerh.).

[3] See footnote 2.

about 1° C. The formulae of Riker and Riker (23) were used in the preparation of all media for spore-germination tests, excepting the malt agar, which was made according to Fischer's (11) formula No. 5.

In the study of the occurrence of species of barley loose smut in the United States, as indicated by epispore and spore-germination characters (p. 197), the former were determined by examination under an oil-immersion lens. Spore-germination characters were determined by sowing the spores on fresh 2 per cent potato-dextrose agar in 50-mm. Petri dishes. The cultures were then incubated at 20° C. in a constant-temperature chamber, and examined after 6 to 12 hours. The spores were sown according to a previously-described method (33) whereby individual spores in aqueous suspension were uniformly distributed over the agar (Fig. 1). Every spore was placed in direct contact with the exposed surface of the substrate where it could be accurately observed for type of germination. It was observed, for example, that before this method was developed, if a small clump of dry spores of *Ustilago hordei* or *U. nigra* were placed on potato-dextrose agar, the spores touching the medium produced sporidia; those piled on other spores, however, sometimes germinated by producing only mycelial threads. The two different conditions involved were responsible for this, but the spores mistakenly presented a mixed germination on one medium. The technique of setting up the cultures, therefore, is an important consideration in an accurate species determination.

## EXPERIMENTAL RESULTS

### Groups of Barley Loose Smut in the United States

As noted previously (p. 196), *Ustilago nuda, U. nigra,* and the so-called *U. medians* have in common the production of the loose type of smutted head and of echinulate chlamydospores. The morphological character distinguishing the three species is the mycelial germination of the chlamydospores of *U. nuda,* the sporidial germination of those of *U. nigra,* and the mixture of mycelial and sporidial types produced by the chlamydospores of *U. medians.* Heretofore, however, it has been difficult to judge the distribution and importance of these species in this country because, in some of the articles, the binomial *U. medians* clearly was used not in reference to a smut with the mixed *U. nuda-U. hordei* type of germination, as described for that species, but to one with the sporidial type of germination. In other reports, the implications of this binomial are not given, and it may or may not have been used in compliance with Biedenkopf's description. As a result of the investigation here recorded, the fungi of the 500 loose-smut collections examined were assignable to 4 well-defined groups on the basis of (1) the type of smutted heads, (2) epispore markings, and (3) type of spore-germination on 2 per cent potato-dextrose agar at 20° C. The defining characters and number of collections found in each group follows:

Group 1.—Loose-smut heads; echinulate spores that produce only mycelial threads (Fig. 1, A). 192 collections. This is the type of smutted head, epispore

marking, and chlamydospore germination described for *Ustilago nuda* Rostrup (24).

Group 2.—Loose-smut heads; echinulate spores that produce only promycelia and sporidia (Figs. 1, B, 2 and 4). 209 collections. This is the type of smutted head, epispore marking, and chlamydospore germination described for *Ustilago nigra* Tapke (29, 30).

Group 3.—Loose-smut heads; echinulate spores. An indefinite number of the spores produce mycelial threads, while the remainder simultaneously produce promycelia and sporidia (Fig. 1, C). 93 collections. This is the type of smutted head, epispore markings, and chlamydospore germination described for *Ustilago medians* by Biedenkopf (4).

Group 4.—Heterogeneous forms having a mixture or blending of characters of smutted heads, or epispore markings of *Ustilago nigra* and *U. hordei* Lagerh.[4] Spores produce only promycelia and sporidia. 6 collections. The existence of this small group must be recognized, but the variability in smutted head and epispore characters hardly permits taxonomic treatment. They probably are hybrid forms.

Of the 500 collections studied, 192 were pure *Ustilago nuda* (group 1). and 209 were pure *U. nigra* (group 2). Chlamydospores of each of the 209 collections of *U. nigra* were applied to the surface of seed of Odessa (C.I. 934) barley. This variety proved to be highly susceptible to every collection. All of these collections, therefore, have the characters described for *U. nigra* in the original (29) and supplemental (30) descriptions of this species, *i.e.*, loose-smut heads, echinulate spores that germinate with sporidia, and the ability to produce seedling infection. The 93 collections of group 3 were studied further and the results are given below.

## The Composition of Collections Complying with the Description of Ustilago medians Biedenkopf (Group 3)

A mixture of the normal mycelial and sporidial germinations occurred in each of the 93 collections that appeared to be *Ustilago medians.* Both types developed with great vigor in 6 to 12 hours and complied with the mixed "*U. jensenii-U. hordei*" type of germination described by Biedenkopf (4) for *U. medians.* The proportion of the two types varied widely in the different collections, ranging from a trace of one or the other to approximately equal proportions of each. Further examination of these 93 (group 3 or *U. medians*) collections, revealed that each was simply a mixture of the sporidia-producing *U. nigra* and the mycelium-producing *U. nuda,* rather than an intermediate species. This was proved conclusively in 3 ways: (1) Plants of the smut-susceptible Odessa (C.I. 934) barley were grown from seed coated with chlamydospores of each of the 93 mixed collections. This procedure filtered out the *U. nuda* element due to its inability to produce seedling infection. The *U. nigra* element of each collection, however, produced high percentages of smutted heads whose spores invariably produced only the sporidial type of germination; (2) flowers of Lion (C.I. 923) and Odessa (C.I. 934) barley, both of which are susceptible to *U. nuda* and *U. nigra,* were hand-inoculated with each of 10 of the collections from group 3. On maturing, some of the seed was sown without treatment; another lot was

[4] *Ustilago hordei* Lagerh. (*U. hordei* (Pers.) K. and S.) produces the covered type of smutted head, has smooth chlamydospores, which produce sporidia on 2 per cent potato-dextrose agar at 20° C.

soaked in formaldehyde solution (1:320) for 1 hour before sowing. This treatment does not control *U. nuda,* but it does control *U. nigra* (30). The plants grown from treated seed produced only *U. nuda,* the spores of which yielded only the mycelial type of germination. Plants from the nontreated seed of Lion and Odessa, however, became infected by both *U. nuda* and *U. nigra;* (3) spores of 10 of the mixed collections of group 3 were inserted in the flowers of Atlas (C.I. 4118) and Trebi (C.I. 936) barleys. In the writer's studies, Atlas has proved immune from *Ustilago nigra* and highly susceptible to *U. nuda.* Trebi, however, has been immune from *U. nuda,* but highly susceptible to *U. nigra.* The mature seed from inoculated flowers of both varieties was later sown in a greenhouse. From the smutted heads of Atlas plants only *U. nuda* was recovered, and from Trebi only *U. nigra.* Both *U. nuda* and *U. nigra* have been isolated many times from naturally mixed collections by the foregoing methods and then used separately year after year in studies of physiologic races and in other experiments. Such isolates have never shown evidence of the simultaneous mixed type of spore-germination described by Biedenkopf for *U. medians.*

In the light of the foregoing results, it would seem that Biedenkopf's *Ustilago medians* may have been similar to the numerous field collections of barley loose smut reported herein that proved to be mixtures of *U. nuda* and *U. nigra* and that germinated in full conformity with his description of *U. medians.* It is noteworthy, too, that in germinating random samples of mixtures of *U. nuda* and *U. nigra,* more of either one may be taken in successive samples. The sporidial germination, therefore, may predominate in one sample and the mycelial in the next. Biedenkopf reported results of this kind in his two tests of *U. medians.* Apparently, no other smut has been reported that persistently develops the normal mycelial and sporidial types of germination simultaneously in one culture. No further specimens of *U. medians* as described by Biedenkopf evidently have been found anywhere.

It would seem unsafe to assume that the sporidial element of *Ustilago medians* was identical with *U. nigra;* in fact, the sporidial element may not have been a loose smut. For example, the writer has collected an echinulate-spore, sporidia-forming, barley smut similar to *U. nigra,* except that during the past 5 years it has consistently produced the covered-smut type of head. Spores of this smut were dusted on the surface of Odessa (C.I. 934) barley seed. Earlier, the flowers from which this seed developed were hand-inoculated with spores of the deep-infecting loose smut, *U. nuda.* Plants from this twice-inoculated seed, grown in a greenhouse, produced many smutted heads of different kinds. Several of the loose-smut heads evidently had some admixture of the echinulate-spore covered smut because some spores formed sporidia, while others formed mycelium on 2 per cent potato-dextrose agar at 20° C. The loose-smut heads, echinulate chlamydospores, and mixed types of spore germination, therefore, were like *U. medians,* but the sporidial element in this mixture was not the loose-smut fungus *U. nigra.*

Another possible explanation of Biedenkopf's description might be that his smut was a sporidia-producing species that in both of his tests germinated abnormally to resemble the mixed mycelial-sporidial type. This phase is considered in the following pages.

## Variability in Spore-germination

Commenting on the species validity of *Ustilago medians* and *U. nigra,* Ruttle (25) called attention to the fact that Biedenkopf's germination studies were performed in the heat of July "under which conditions even spores of *U. hordei* may germinate by germ tubes." It is well known that so-called sporidia-producing smuts, *e.g., U. hordei,* do not, on germinating, always produce sporidia; but, in the light of the studies of Hüttig (16), Jones (17, 18), Shih (27), and numerous others, it is equally true that they do not simultaneously produce both the sporidial and mycelial types of germination in optimum development under one set of conditions in culture, as described by Biedenkopf for *U. medians.* However, in view of the relatively limited knowledge of spore germination in *U. nigra,* a study of this character as influenced by different temperatures and substrata was included in the present investigation.

In the temperature studies, spores were sown by a method previously described (33) on the surface of 2 per cent potato-dextrose agar in 50-mm. Petri dishes and incubated in accurately controlled temperature chambers at 5, 10, 15, 20, 25, 30, 35, and 40° C. Three chlamydospore cultures of each of 3 physiologic races of *Ustilago nigra* were incubated at each temperature. The spores were nearly 100 per cent viable. For comparison, the 3 well-known sporidia-producing small-grain smuts, *U. avenae, U. levis,* and *U. hordei* were similarly studied. The results with *U. nigra* were as follows: At 5, 10, 15, 20, and 25 degrees C. all of the viable spores germinated with short tubes and sporidia. At 30° the temperature was obviously too high for normal germination, and the spores uniformly developed short, thick, segmented threads resembling links of sausage. At 35°, only short tubes were formed, and at 40° no germination occurred. The results with *U. avenae, U. levis,* and *U. hordei* were similar to those with *U. nigra* throughout the test. Spore germination in *U. nigra* at various temperatures, therefore, is strikingly like that of *U. avenae, U. levis,* and *U. hordei,* and unlike that described by Biedenkopf for *U. medians.*

In the study of variability in type of spore germination, as influenced by different substrata, the same collections and species used in the foregoing study were again employed. Each was tested in triplicate cultures at 20° C. on the following substrata: 1, On the lower surface of bare glass cover slips suspended on glass bars above a moist blotter in a moisture chamber; 2, in sterile distilled water; 3, in sterile tap water; 4, on plain gelatin; 5, on nutrient gelatin; 6, on 2 per cent potato-dextrose agar; 7, on nutrient agar; 8, on malt agar; 9, on prune agar; 10, on plain distilled-water agar.

Throughout the test, spore germination was strikingly similar in *Ustilago*

*avenae, U. levis, U. hordei,* and the 3 collections of *U. nigra.* The results, moreover, agreed fully with those of Brefeld (5), Hallier (12), Fischer (10), Kulkarni (20), Sartoris (26), Shih (27), Stakman (28), and others in sim-

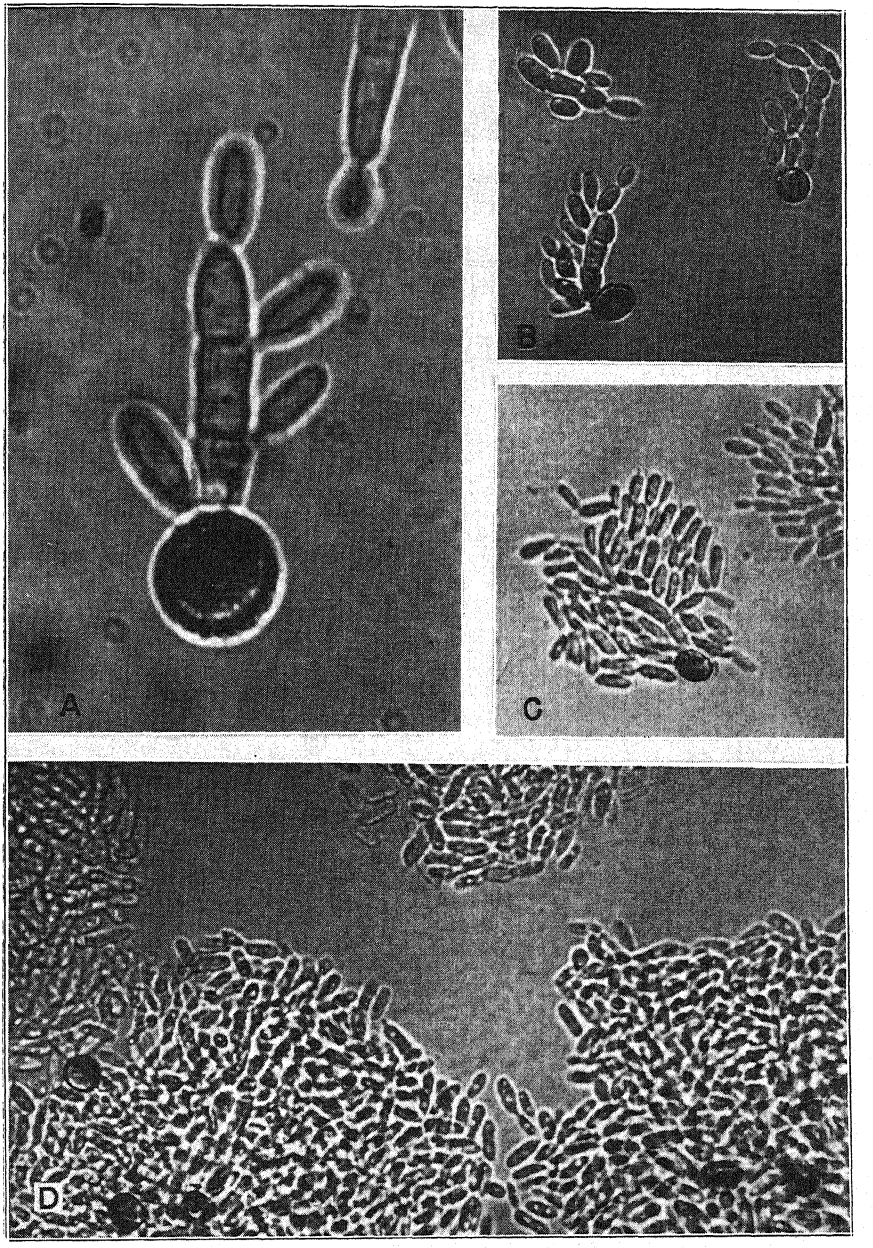



FIG. 2. Germination of spores of *Ustilago nigra* on 2 per cent nutrient-dextrose agar at 20° C. A. An early stage showing the echinulate chlamydospore, biseptate promycelium and four primary sporidia. ×1900. Another promycelium, bearing a young primary sporidium at its apex is shown in the upper-right corner. B, C, and D. Later stages of germination showing multiplication of sporidia. ×600.

ilar studies of various sporidial smuts. On potato-dextrose, nutrient-dextrose, and malt agars the 4 species formed only sporidia. As shown in figure 2, the promycelia were compact and sturdy, and sporidia were produced in countless numbers. On the other hand, when spores of these smuts were

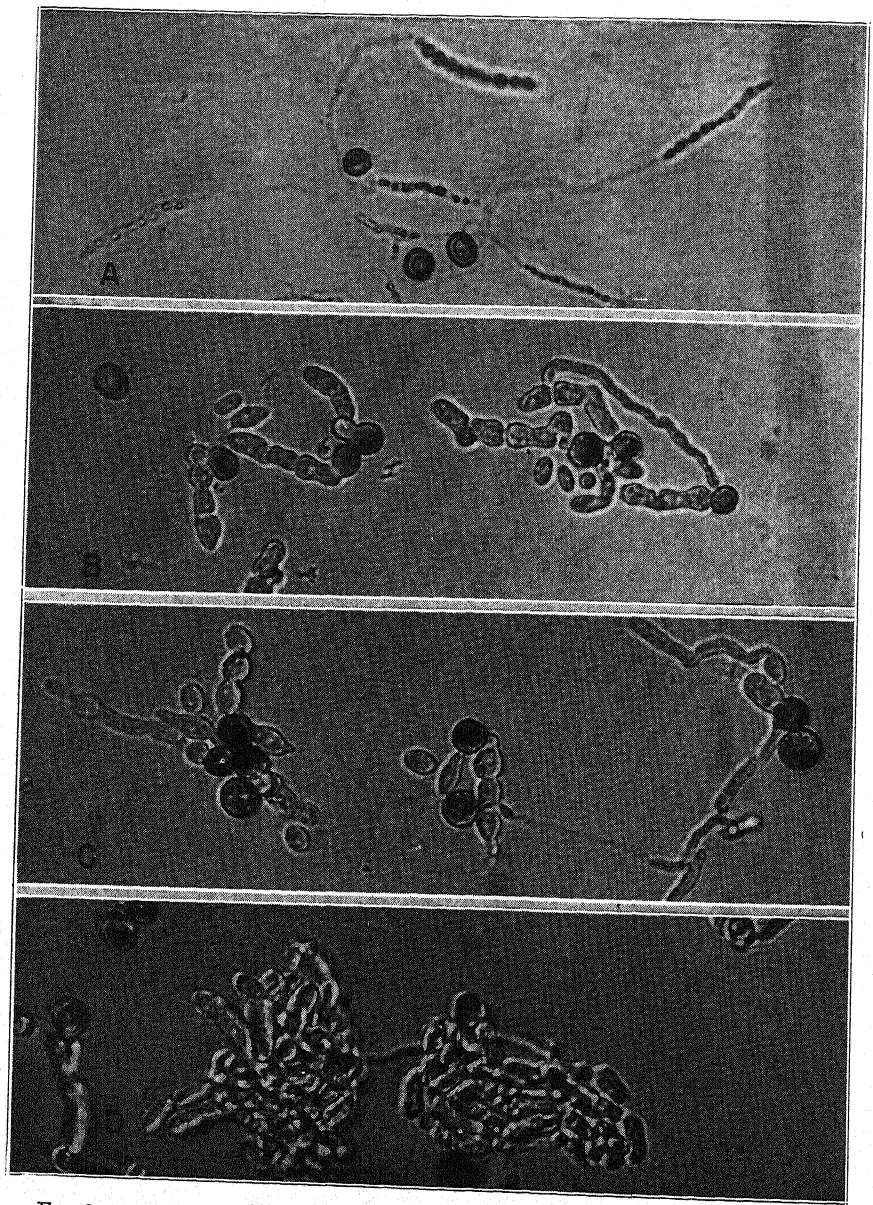



FIG. 3. Examples of the influence of different conditions on spore germination of *Ustilago avenae, U. hordei, U. levis* and *U. nigra*. A. *U. levis* on a bare glass cover slip in humid air. B. *U. hordei* on distilled-water agar. C. *U. nigra* on distilled-water agar. D. *U. avenae* on plain gelatin. All photographs taken after 5 days in culture at 20° C. ×600.

FIG. 4. A. An unsmutted head of Odessa (C.I. 934) barley. B, C, E. Odessa heads smutted with *Ustilago nigra* as they appeared, respectively, 1 day after emergence, when the barley was in the milk stage, and when the barley was ripe. D. A smutted head of Himalaya (C.I. 1312) barley showing the greater persistence of awns in smutted heads of this strongly awned variety. F. Smutted head of the hooded, hulless, Nepal (C.I. 595) barley. The distinguishing head characters of different barley species and varieties are largely lost when the heads become smutted. ×1.

germinated in the absence of nutriment on bare glass cover slips in a moisture chamber, only mycelial threads were produced (Fig. 3, A). Prune agar was fairly favorable for the production of sporidia. Plain and nutrient gelatins were clearly unfavorable. The spores gave rise mostly to abortive, compact masses of cells (Fig. 4, D). Biedenkopf used gelatin and diluted plum decoction in the study of spore germination in *U. medians,* but did not give the compositions of these media. It is evident, however, that on plain gelatin, nutrient gelatin, and prune agar, spore germination in *U. nigra* bears no resemblance to the mixed mycelial-sporidial type (Fig. 1, C) described by Biedenkopf for *U. medians.* Distilled-water agar also was unfavorable (Fig. 3, B, C). On this medium, and also in tap water and distilled water, some spores produced a few sporidia, others a few short threads, while still others developed a third type, not mentioned by Biedenkopf, usually consisting of two branches, one bearing a few sporidia, the other forming an abortive mycelium-like growth. It was thus possible to produce in *U. nigra, U. hordei, U. avenae,* and *U. levis* an abnormal or atypical sporidial germination that might be taken to resemble a mixture of the mycelial and sporidial types accompanied by a third type, as described above. This germination, however, was very slow, and, after an abortive development, came to a standstill. Cultures several weeks old still showed very scant growth. In morphology and rate and degree of development, therefore, this abnormal sporidial type unmistakably was not the luxuriant development of robust mycelial threads and thousands of sporidia, budding like yeast, that Biedenkopf described for *U. medians,* and first suspected to be "*Ustilago Hordei* und *Ustilago Jensenii* . . . zusammen" (4). It should be noted, moreover, that Biedenkopf obtained the mixed mycelial-sporidial germination of *U. medians* in only 4 hours. It is evident from studies of other small grain smuts (3, 16, 27) that this is a very rapid rate, indicating that optimum or near optimum temperatures existed during germination. At such temperatures and on favorable substrata such as Biedenkopf evidently used, neither a mycelial nor a sporidial smut alone would develop the mixed mycelial-sporidial type of germination described for *U. medians* (4). But, granted even that *U. medians* had been based on the abnormal germination of a sporidial smut, the species name would have to be rejected in compliance with Article 65 of the International Rules of Botanical Nomenclature (7), which reads "A name or epithet must be rejected when it is based on a monstrosity."

In view of the foregoing results with different temperatures and substrata, it is evident that, even when variability in spore germination is given the widest latitude, the persistent, normal development of two distinctly different types of spore germination with which Biedenkopf characterized *Ustilago medians,* does not occur in *U. nigra* and other sporidial smuts. *Ustilago nigra* and *U. medians,* therefore, are not synonymous species.

As shown recently by Christensen and Rodenhiser (8), the genetic makeup of the chlamydospore, as well as its environment, may be concerned

in variability of chlamydospore germination. In *Ustilago zeae* and *U. levis,* for example, certain lines consistently produce the mycelial instead of the normal sporidial type of germination. In *U. nigra,* however, such lines apparently have not been found or reported, although many specimens of the fungus have been studied in recent years.

## Variability in Ustilago nigra

Variation occurs in *Ustilago nigra* in prominence of the chlamydospore echinulations, intensity of the dark brown color of the spore mass, compactness and powderiness of the smutted heads, degree to which the fungus destroys the awns of smutted heads (Fig. 4) etc. The host variety, physiologic race of the smut, and the conditions under which the plants are grown seem largely responsible for these variations (32). Similar or greater variability than that yet found in *U. nigra* has been reported in such well-known and long-established species as *U. avenae* and *U. levis* (14), *U. hordei* (32), *Sphacelotheca sorghi* and *S. cruenta* (36), *Tilletia tritici* and *T. levis* (15), and others (8). Ruttle (25) erected "types" of barley smut on small morphological differences like those noted above for *U. nigra,* but they fail to breed true and apparently serve no useful taxonomic purpose.

## Conclusions and Amplified Description of Ustilago nigra

In the light of the foregoing facts, the following conclusions seem logical:

1. The so-called *Ustilago medians* evidently does not occur in the United States. *Ustilago nuda* and *U. nigra,* however, frequently occur together in the barley fields of this country and a mixture of the spores of these two species germinates by producing the mixture of mycelial and sporidial types, as described for *U. medians,* indicating that the latter probably was based on a mixture. 2. Excepting occasional hybrid types, loose smut of barley in this country is caused only by *U. nuda* and *U. nigra.* 3. The incidence of species in the present study indicates that *Ustilago nigra* has become as widespread as *U. nuda* in the United States. It is, therefore, now possible through simple, inexpensive seed treatments, to save half of the estimated loss from loose smut averaging 2 million bushels of barley annually (29). 4. When *U. nuda* and *U. nigra* are uniformly germinated on 2 per cent potato-dextrose agar at 20° C., *U. nuda* develops only the mycelial type of germination and *U. nigra* only the sporidial type. These 2 species, therefore, always may be readily and accurately identified by the striking differences in this character (Fig. 1, A and B). 5. The normal germination of *U. nigra* is consistently that of a true sporidial smut; it positively does not resemble "*Ustilago Hordei* and *Ustilago Jensenii* . . . zusammen,"[5] as described for *U. medians* by Biedenkopf (4). 6. Through the use of different temperatures or different substrata, chlamydospores of *U. nigra,* like those of *U. avenae, U. levis,* and *U. hordei* may, on germination in culture, produce only sporidia, or mycelia only, or a simultaneous development of abortive forms

[5] See footnote 2.

of both sporidia and mycelia. The simultaneous, optimum, development of both the mycelial and sporidial types in one culture, as described by Biedenkopf, for *U. medians* did not occur. 7. In view of these facts it is evident that, even when variability in spore germination is given the widest latitude, identity of *U. nigra* is distinct from that of the mycelial-sporidial *U. medians*. *Ustilago nigra*, therefore, is entitled to rank as a species. 8. The binomial *U. medians* Biedenkopf must be rejected (A) because it was obviously based on a mixture of two different fungi, erroneously supposed to form part of one individual; (B) because it cannot be applied with certainty to any known fungus causing loose smut of barley; and (C) because the binomial has become a source of confusion or error due to its use with different meanings. It, therefore, falls clearly within the scope of articles 64, 63, and 62 of the International Rules of Botanical Nomenclature (7), which are as follows: Article 64.—"A name of a taxonomic group must be rejected if the characters of that group were derived from two or more entirely discordant elements, especially if those elements were erroneously supposed to form part of the same individual" (*Nomina confusa*). Article 63.—"A name of a taxonomic group must be rejected when its application is uncertain" (*Nomen dubium*). Article 62.—"A name of a taxonomic group must be rejected if owing to its use with different meanings it becomes a permanent source of confusion or error" (*Nomina ambigua*).

## Amplified Description of Ustilago nigra

*Ustilago nigra* was briefly described in 1932 (29), and further described in 1935 (30) and in 1941 (33). In order to summarize these descriptions, the following amplified description is presented:

*Ustilago nigra* Tapke. Sori in spikelets forming dusty, dark-brown spore masses 3–5 × 5–10 mm. Sori temporarily protected by a thin membrane, but soon becoming dissipated, leaving the naked rachis (Fig. 4, B, C, E). Spores echinulate, of lighter color on one side, spherical to subspherical, 5.5–7.5 × 6–8 μ, mostly 6.5 × 7 μ, forming a 3-cellular promycelium bearing typically 4 lateral sporidia when germinated on 2 per cent potato-dextrose agar and similar media at about 20° C. (Fig. 2, A, B, C, D).

On *Hordeum vulgare* (31) *H. distichon* (31), *H. intermedium* (31), *H. deficiens* (31), *H. nodosum* (10), *H. pusillum*,[6] *Elymus canadensis* (10), and *Sitanion jubatum* (10). Collected in the following States: Arkansas, Colorado, Delaware, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maryland, Michigan, Minnesota, Mississippi, Missouri, Montana, Nebraska, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Virginia, Washington, West Virginia, Wisconsin, and Wyoming.

*Herbarium Material:* Specimens deposited in the Mycological Collections

[6] Collected by I. M. Atkins, Denton, Texas, and determined by the writer as *Ustilago nigra*, physiologic race 4.

of the Bureau of Plant Industry, United States Department of Agriculture at Washington, D. C. Type No. 71357 on *Hordeum vulgare.*

Soris in spiculis, massam pulverulentam atro-brunneam 3–5 mm. latem, 5–10 mm. longam formantibus, primum membrans tenui tectis, demum nudis; Sporis echinulatis, sphaericis v. subsphaericis 5.5–7.5 μ latis, 6–8 μ longis, plerumque 6.5 μ latis, 7 μ longis; promycelio 3-septato, sporidia 4, lateraliter gerenti.

Hab. in spiculis *Hordei* spp., *Elymi canadensis,* et *Sitanii jubati.* United States.

## SUMMARY

In a study of the different kinds of barley loose smut in this country, 500 specimens collected from 33 States were uniformly germinated on 2 per cent potato-dextrose agar at 20° C. Six of the collections were heterogeneous types. Of the remainder, all of which had the loose type of smutted head and echinulate spores, 192 produced the mycelial germination of *Ustilago nuda,* 209 the sporidial germination of *U. nigra,* and 93 produced on germination a mixture of the normal mycelial and sporidial types and otherwise conformed to the description of *U. medians* Biedenkopf. All of the 93, however, proved to be simply mixtures of the mycelium-producing *U. nuda* and the sporidia-producing *U. nigra.* A smut conforming to the description of *Ustilago medians* apparently does not occur in the United States nor elsewhere.

When spores of *Ustilago nuda* and *U. nigra* are uniformly germinated on 2 per cent potato-dextrose agar or other suitable media at usual room temperatures, the former consistently develops only mycelial threads, and the latter always forms only promycelia and sporidia. The spore-germination test, therefore, quickly and accurately shows (1) whether the causal fungus is *U. nuda, U. nigra,* or a mixture of the two, and (2) the type of seed treatment necessary for control.

In a comparative study, chlamydospores of *Ustilago nigra, U. avenae, U. levis,* and *U. hordei* were germinated on 10 different substrata at 20° C. and on 2 per cent potato-dextrose agar at 5, 10, 15, 20, 25, 30, 35 and 40 degrees C. Conclusive evidence was obtained that *U. nigra* is a true sporidia-producing fungus like *U. avenae, U. levis,* and *U. hordei.*

The incidence of species in the present study indicates that *Ustilago nigra* has become as widespread as *U. nuda* in the United States. It is, therefore, possible through simple, inexpensive seed treatments, to prevent half of the estimated annual 2-million-bushel loss attributed to barley loose smut.

It is concluded that, with the exception of occasional hybrid types, such as are found also in wheat and oat smuts, loose smut of barley in the United States is caused by *Ustilago nuda* or *U. nigra;* that these two species may be readily distinguished through a simple spore-germination test and in other ways; that *U. nigra* is a valid species; and that *U. medians,* appar-

ently, was erroneously based on a mixture of two different smut fungi. The binomial, therefore, must be rejected.

An amplified description of *Ustilago nigra* is given.

DIVISION OF CEREAL CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY STATION,
U. S. DEPARTMENT OF AGRICULTURE,
BELTSVILLE, MARYLAND.

## LITERATURE CITED

1. ALLISON, C. C. Hybridization between *Ustilago hordei* and *U. medians*. Phytopath. 25: 5. 1935.
2. ————. Studies on the genetics of smuts of barley and oats in relation to pathogenicity. Minn. Agr. Exp. Stat. Tech. Bull. 119. 1937.
3. APPEL, O., and E. RIEHM. Die Bekämpfung des Flugbrandes von Weizen und Gerste. Arb. K. Biol. Anst. Land. u. Forstw. 8: 343–426. 1911.
4. BIEDENKOPF, H. *Ustilago medians* ein neuer Brand auf Gerste. Zeitschr. f. Pflanzenkr. 4: 321–322. 1894.
5. BREFELD, O. Recent investigations of smut fungi and smut diseases. (Translated by Erwin F. Smith.) Jour. Mycol. 6: 1–8, 59–71, 153–164. 1890.
6. ————. Untersuchungen aus dem Gesammtgebiete der Mykologie. Brandpilze III 12: 99–227. (Münster) 1895.
7. BRIQUET, J. International rules of botanical nomenclature. 151 pp. (Gustav Fischer, Jena) 1935.
8. CHRISTENSEN, J. J., and H. A. RODENHISER. Physiologic specialization and genetics of the smut fungi. Bot. Rev. 6: 389–425. 1940.
9. DICKSON, J. G. Outline of diseases of cereal and forage crop plants of the northern part of the United States. 259 pp. Burgess Publ. Co. (Minneapolis) 1939.
10. FISCHER, G. W. Studies of the susceptibility of forage grasses to cereal smut fungi. II. A preliminary report on *Ustilago hordei* and *U. nigra*. Phytopath. 29: 490–494. 1939.
11. ————. Fundamental studies of the stripe smut of grasses (*Ustilago striaeformis*) in the Pacific Northwest. Phytopath. 30: 93–118. 1940.
12. HALLIER, ERNST. Beweis, dass der Cryptococcus keimfähig und von höheren Pilzformen abhängig ist und Widerlegung der Ansichten der Bary'schen Schule über Bierhefe. Zeitschr. f. Parasitenk. 3: 217–244. 1872.
13. HERZBERG, P. Vergleichende Untersuchungen über landwirtschaftlich wichtige Flugbrandarten. Beit. z. Physiol. u. Morphol. nied. Org. 5: 1–36. 1895.
14. HOLTON, C. S. Further studies on oat smuts, with special reference to hybridization, cytology and sexuality. Jour. Agr. Res. [U. S.] 62: 229–240. 1941.
15. ———— and H. A. RODENHISER. New physiologic races of *Tilletia levis* and *T. tritici*. Phytopath. 32: 117–129. 1942.
16. HÜTTIG, W. Über den Einfluss der Temperatur auf die Keimung und Geschlechtverteilung bei Brandpilzen. Zeits. Bot. 24: 529–557. 1931.
17. JONES, E. S. Influence of temperature, moisture, and oxygen on spore germination of *Ustilago avenae*. Jour. Agr. Res. [U. S.] 24: 577–591. 1923.
18. ————. Influence of temperature on the spore germination of *Ustilago zeae*. Jour. Agr. Res. [U. S.] 24: 593–597. 1923.
19. KELLERMAN, W. A., and W. T. SWINGLE. Report on the loose smuts of cereals. Kans. Agr. Exp. Stat., Ann. Rpt. (1889): 213–288. 1890.
20. KULKARNI, G. S. Smuts of Jowar (sorghum) in the Bombay Presidency. Agr. Res. Inst. Pusa Bull. 78: 1918.
21. LEUKEL, R. W. Factors affecting the development of loose smut in barley and its control by dust fungicides. U. S. Dept. Agr. Tech. Bull. 293, 19 pp. 1932.
22. ————. Further experiments on the control of barley smuts. U. S. Dept. Agr. Tech. Bull. 513, 11 pp. 1936.
23. RIKER, A. J., and REGINA S. RIKER. Introduction to research on plant diseases. 117 pp. John S. Swift Co. (St. Louis). 1936.
24. ROSTRUP, E. Oversigt over de i 1888 indløbne forespørgsler angaaende sygdomme hos kulturplanter. Tidsskrift for Landøkonomi 8 (Series 5): 744–751. 1889.
25. RUTTLE, M. L. (MRS. NEBEL). Studies on barley smuts and on loose smut of wheat. N. Y. (Geneva) Agr. Exp. Stat. Tech. Bull. 221, 39 pp., 1934.
26. SARTORIS, G. B. Studies in the life history and physiology of certain smuts. Amer. Jour. Bot. 11: 617–647. 1924.

27. SHIH, L. Über den Heterothallismus des Staubbrandes, *Sphacelotheca cruenta* (Kühn) Potter, der Mohrenhirse, *Andropogon sorghum*. Brot. Arch. Mikrobiol. 9: 167–192. 1938.
28. STAKMAN, E. C. Spore germination of cereal smuts. Minn. Agr. Exp. Stat. Bull. 133, 77 pp. 1913.
29. TAPKE, V. F. An undescribed loose smut of barley. Phytopath. 22: 869–70. 1932.
30. ———. A study of the cause of variability in response of barley loose smut to control through seed treatment with surface disinfectants. Jour. Agr. Res. [U. S.] 51: 491–508. 1935.
31. ———. Pathogenic strains in *Ustilago nigra*. Phytopath. 26: 1033–1034. 1936.
32. ———. Studies on the natural inoculation of seed barley with covered smut (*Ustilago hordei*). Jour. Agr. Res. [U. S.] 60: 787–810. 1940.
33. ———. A technique for identifying the loose smuts of barley. Phytopath. 31: 284–286. 1941.
34. THREN, R. Gewinnung und Kultur von monokaryotischen und dikaryotischen Mycel. Ein Beitrag zur Physiologie und Genetik des Gerstenflugbrandes (*Ustilago nuda* (Jens.) Kell. et Sw.). Zeitsch. Bot. 31: 337–391. 1937.
35. TISDALE, W. H., and V. F. TAPKE. Infection of barley by *Ustilago nuda* through seed inoculation. Jour. Agr. Res. [U. S.] 29: 263–284. 1924.
36. ———, L. E. MELCHERS, and H. J. CLEMMER. Strains of kernel smuts of sorghum, *Sphacelotheca sorghi* and *S. cruenta*. Jour. Agr. Res. [U. S.] 34: 825–838. 1927.

# AN OUTBREAK OF PLANTAGO VIRUS IN BURLEY TOBACCO[1]

W. D. Valleau and E. M. Johnson

(Accepted for publication July 30, 1942)

During the meeting of the New England Division of the American Phytopathological Society in 1941, F. O. Holmes showed to one of the writers plants of *Plantago major,* which he stated were affected by a virus similar to the tobacco-mosaic virus because it withstood drying, and when purified and photographed, was indistinguishable in appearance from the tobacco-mosaic virus. The details of his study have since been published (2). He also reminded us of a report, in the 1930 Annual Report of the Kentucky Experiment Station, of a virus of *Plantago major* that we had transferred to tobacco. In these early studies a mosaic-affected *Plantago major* leaf was collected from the experiment station lawn and a transfer made to Turkish tobacco, where a disease developed, different from that produced by any of the strains of the tobacco-mosaic virus then under investigation. On seedling potato leaves the plantago virus caused chlorotic spots that soon became necrotic (3). In this respect it was similar to but not identical with tobacco-mosaic virus. It caused local chlorotic spots on petunia, with chlorosis and distortion in growing-point leaves. No infection followed inoculation of cucumber. On June Pink tomato the plantago virus caused a mild mottling which might have been easily overlooked. In addition it caused necrosis of the underside of the midveins. Dried tobacco material was saved to determine whether the virus withstood drying but this material was not tested until recently when both tobacco mosaic and plantago virus were isolated from it. On Cut-short, Striped creaseback, and White creaseback beans the plantago virus produced no necrotic spots, while tobacco-mosaic virus strains caused local necrotic spotting. Primarily because of this fact, it was concluded that the plantago virus was not a strain of tobacco-mosaic virus, and probably was of little importance to the tobacco industry. No further study of the virus was made at that time.

After returning to Lexington from the meeting, the writers examined plants of plantago and found many infected with a virus. On inoculated Ky. 16 burley this virus caused local chlorotic spots, followed eventually by necrosis of the whole inoculated area; it also caused vein-clearing of growing-point leaves, soon followed by mottling in which the dark green areas were small specks, instead of large, irregular areas, as in tobacco mosaic. There was some distortion, and necrotic spots developed in leaves or portions of leaves that were young enough to be rapidly invaded, but too old to develop mottling at the time of invasion (Fig. 1, B). The virus is similar in this respect to burning strains of the tobacco-mosaic virus (5), except that the latter rarely cause burning in the greenhouse. In older plants, growing in the ground bench, the patterns were usually larger and distortion less than

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

occurs with many strains of tobacco mosaic. On Turkish tobacco, symptoms developed similar to those recently described by Holmes (2). The plantago virus, as it occurs in Kentucky, is obviously the same as that found in eastern United States and described by Holmes.

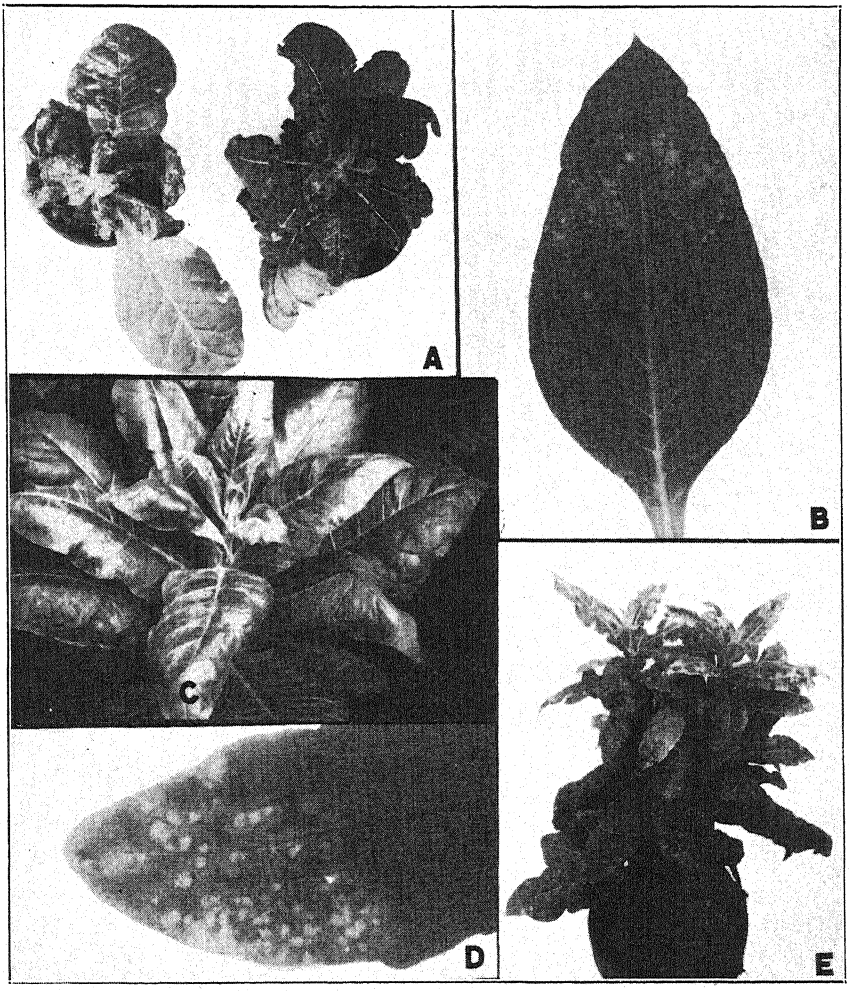



FIG. 1. A. Two N′N′ burley plants, Kelley (left) and Pepper, infected with the plantago virus from plantago. The plant on the left was inoculated 51 days later than the one on the right, and shows the severe necrotic reaction following inoculation. Photographed 36 days after the Kelley plant was inoculated. B. A leaf from a plant of Ky. 16 burley inoculated with the plantago virus, showing necrotic areas in invaded tissue too old to develop mottle, and speckled mottling in the younger portion of the leaf. C. Ky. 16 burley naturally infected with the plantago virus in the field. This shows the chlorotic response of the virus in an n′n′ plant. D. *Plantago major* leaf 19 days after inoculation with a variant of tomato aucuba mosaic virus. The patterns are somewhat more prominent than those produced by the ordinary strains of the tobacco-mosaic virus. Leaf print on direct positive paper. E. Ky. 36–12 N′N′ plant from Mr. Bell's field infected with the plantago virus, transplanted to the greenhouse in August and photographed November 6, 1941.

Late in August, 1941, R. E. Bell, a neighboring farmer, brought us several plants of Ky. 36–12 burley tobacco affected with a virus disease we did not recognize. The plants were markedly stunted; had long, depressed, dark-colored streaks on the stalks, and discolored patches in the pith which sometimes had concentric patterns. The leaves had yellow blotches, sometimes only 2 or 3 to the leaf, which later became necrotic (Fig. 1, E). Some leaves had necrotic line patterns and, on the undersides of the midveins, necrotic areas. The disease differed from tobacco streak and ring spot because there was no indication of so-called recovery in the new growth. The farmer said that the disease had been present since shortly after setting the crop. A count in the field indicated that about 2 per cent of the plants were affected but the farmer claimed this percentage was low because he had destroyed many affected plants during the summer. A crop of Ky. 5 burley, plants for which were obtained from a neighbor's bed and set after Ky. 36–12 in the same field, was entirely free from the disease, suggesting that infection of Ky. 36–12 probably originated in the plant-bed. The only disease of tobacco, similar to the Bell disease, which we had seen in the past, was one collected in Marion County, Kentucky, in 1923, and illustrated under the name "streak" in Kentucky Bulletin 328. A re-examination of records and photographs of this disease indicates that it corresponds more closely to the Bell disease than to the disease now commonly known as tobacco streak.[2]

Infected plants from the Bell field were transferred to the greenhouse where they continued to show a streak type of disease month after month (Fig. 1, E). Inoculations were made from these transplanted plants to Burley 16 and to Turkish, where a mottle, rather than a necrotic, disease developed. A comparison of these mottled plants with Turkish and Burley 16 plants inoculated from plantago, indicated that the diseases were at least very similar, and the suggestion occurred that the virus causing the streak-like disease in the field was the plantago virus in a variety of tobacco, which reacted to the virus in essentially the same way as burley tobacco plants containing the N factor from *N. glutinosa* react to any of the strains of the tobacco mosaic virus.

It had previously been shown that the varieties of burley and dark tobaccos grown in Kentucky, as well as collections from other parts of the world, could be divided into two distinct groups, based on their reaction to white tobacco mosaic, tomato aucuba mosaic, and to several other collections of the tobacco-mosaic virus (6). On one group of varieties, these viruses caused necrotic lesions in about 48 hours, and streak, if the virus became systemic. The same group inoculated with ordinary strains of the virus always developed the usual systemic mottle. The other group when inoculated with the necrotic strains of the virus develop typical mottle mosaic. The gene controlling necrotic spotting is a simple dominant, as is the similar gene in *Nicotiana glutinosa*. The necrotic spotting gene in the common tobacco varieties will be designated N′ to distinguish it from the N gene from

[2] In 1942 the Marion county farm was visited and numerous infected plantains were found bordering the field in which the disease of tobacco was found in 1923.

*N. glutinosa,* which has now been transferred to several varieties of tobacco. Whether or not the N and N′ genes are allelomorphs has not been determined. Burley tobacco varieties in which necrotic spotting genotypes occur, and which will be designated as N′ burleys, are Kelley, Judy's Pride, Pepper, and Ky. 36–12. Dark varieties that contain the N′ factor are One Sucker, Improved Smith, Rudolph's Improved Mammoth, Madole, Brownleaf, and Ambalema. The following varieties develop typical mosaic when inoculated with the necrotic strains of the virus: Turkish, Ky. 5, Ky. 16, Gay's Yellow, and Chileno Corentino.

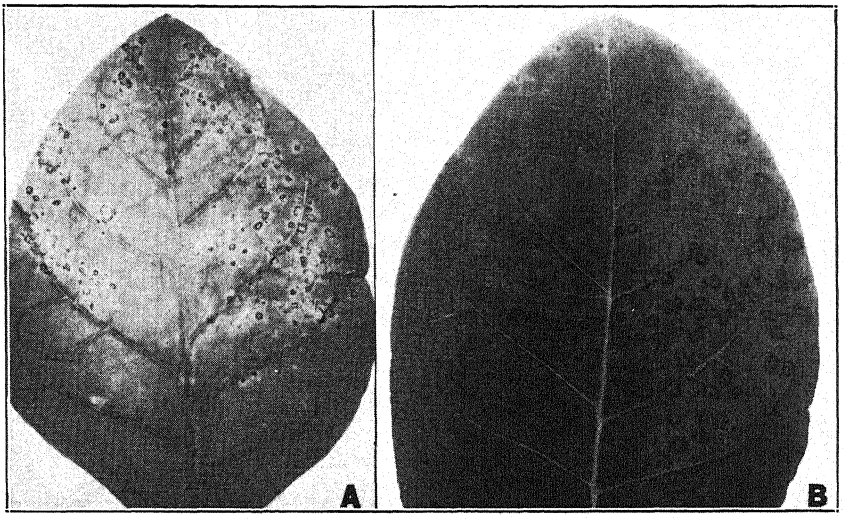



FIG. 2. A. Plantago virus collected on Ky. 16 burley (Fig. 1, C), transferred to *Plantago major* where it caused systemic infection, and then to Kelley burley (above) where it produced the necrotic reaction. Print from leaf onto direct positive paper. B. A leaf of an NN′ burley plant ($F_1$ Pepper N′N′ × Ky. 48–7 NN) inoculated on the left side with plantago virus and the right with tobacco mosaic virus. The N′ gene appears to be dominant over the N gene as to the plantago virus.

To test the hypothesis that Bell's field disease was caused by the plantago virus, and that the plantago virus would prove to be a necrotic spotting virus in N′ burleys, the Bell farm was visited again with the following results: Prior to 1941 the grower had placed his tobacco bed in the plowed field where the tobacco was to be grown, and had had little if any trouble from this disease. In 1941 he decided to place his beds, then and thereafter, in a grass plot surrounding his residence, which in places was found to be nearly a continuous mat of *Plantago* sp. Seven collections of plantago, 2 from the plant bed and 5 from the grass plot, proved to be infected with a virus. The plant bed had been mowed but suckers were growing from the tobacco stubs, several of which had a disease identical with the field disease. Transfers from plantago and from necrotic tobacco plants in the bed to Turkish tobacco and Ky. 16 produced identical symptoms, entirely different from the field disease but identical with the disease caused earlier by the plantago virus in these

varieties of tobacco. Transfers of the viruses from all sources were then made to plants of N′ burley varieties, Pepper and Kelley, and to an N′ dark variety, Ky. 129. On these varieties necrotic spots, of the type shown in Fig. 2, A, developed in about 48 hours on all inoculated leaves. The virus was localized in the inoculated leaves in 76.6 per cent of the 160 plants inoculated but in 24.4 per cent the virus became systemic, producing a disease entirely similar to the necrotic field disease (Fig. 1, A). It seemed evident from these results that the plantago virus, which produces a mottle disease in Turkish and Ky. 16, caused the streak-like disease of Ky. 36–12 in the Bell field.

On July 14, 1941, a virus-diseased plant, which looked as though it were affected by a strain of cucumber mosaic, was found in a field of Ky. 16 tobacco. A photograph was made (Fig. 1, C) and the virus transferred to a tobacco plant in the greenhouse. This virus gave all the reactions of the plantago virus, including systemic infection in *Plantago major;* no necrotic lesions in Scotia beans; the mottle reaction in Ky. 16, and the necrotic reaction in N′ burleys (Fig. 2, A). It may, therefore, be concluded that plantago virus in the field, as well as in the greenhouse, causes 2 distinct diseases—streak in N′ varieties and mottle in n′ varieties.[3]

### DISTRIBUTION OF PLANTAGO VIRUS IN N′ TOBACCO PLANTS

When the plantago virus is inoculated to N′ burley or N′ dark tobacco plants, it may be localized in inoculated leaves or it may become systemic. When it becomes systemic, virus particles are carried to various parts of the growing points, where at least a few multiply, producing necrotic spots if the tissues are relatively old, or chlorotic spots that slowly enlarge and with age gradually become necrotic. In studies on the virus distribution in mosaic-resistant plants, it was demonstrated, through the use of bleaching strains of the virus, that virus distribution within the leaf could be determined fairly accurately by the patterns in the leaf. Patternless or green areas were virus-free, but chlorotic areas contained virus (4). Similar tests were made to determine virus distribution in diseased plants of Ky. 36–12, transplanted from the Bell farm to the greenhouse, and in plants of Pepper burley, both of which contain the N′ factor, in comparison with virus concentration in Ky. 16, which regularly becomes mottled. For this purpose 0.6-cm. disks were cut from isolated chlorotic areas and from green areas of N′ plants and from mottled areas of Ky. 16, crushed in 2 drops of M/10 disodium phosphate and rubbed with a glass spatula over as much leaf surface of an N burley plant as it would cover. Counts were made of the total spots on this area. The results are given in table 1. A high concentration of virus was found in the chlorotic and necrotic spots on the leaves of N′ plants, but green areas on the same leaves appeared to be virus-free, except in one in-

[3] In 1942 Mr. Bell used the same plant bed but substituted Ky. 16 n′n′ for Ky. 36–12 N′N′. When the bed was examined June 9, a few plants were affected by a mottle mosaic that proved to be caused by the plantago virus, and, later, many mottled plants affected with the plantago virus were found in the field.

stance. Thus, although the virus becomes systemic in N′ plants, it is limited in its distribution to a relatively few well-defined chlorotic or necrotic spots. Virus concentration in the mottled leaves of Ky. 16, inoculated with plantago virus, was high. There appeared to be no localization of the virus whatever in invaded leaves of Ky. 16.

TABLE 1.—*Virus distribution in chlorotic and necrotic areas and in green areas of N′ burley plants inoculated with the plantago virus, as indicated by the number of necrotic spots produced on N burley leaves*

| Virus source | Disk from | Total disks | Total spots on N burley | Av. No. spots per disk |
|---|---|---|---|---|
| Ky. 36–12 Bell's field | Pepper burley, necrotic and chlorotic areas | 18 | 3,779 | 210 |
| Same | Same—green areas | 17 | 0 | 0 |
| | | 1 | 34 | 34 |
| Plantago, 2 strains | Same—chlorotic and necrotic areas | 48 | 15,756 | 328 |
| Plantago, 2 strains | Same—green areas | 18 | 0 | 0 |
| Plantago, 3 strains | Plantago leaves | 18 | 6,957 | 386 |
| Plantago and tobacco, 12 strains | Ky. 16 | 12 | 5,347 | 445 |
| White tobacco mosaic | Ky. 16 | 6 | 1,909 | 318 |
| Yellow tobacco mosaic | Ky. 16 | 6 | 1,653 | 275 |

## REACTION OF PLANTAGO VIRUS ON NN′ PLANTS

Tobacco-mosaic virus strains and the plantago virus both produce essentially the same type of necrotic spots on NN Burley 48–7, derived from Holmes' fifth backcross of Ky. 16 on *N. digluta*. On N′N′ burley the plantago and a few strains of the tobacco-mosaic virus produce necrotic spots, but most tobacco-mosaic virus strains cause only mottle. The spots on young, tender N′N′ plants are very similar to spots on NN plants, but on older plants the spots develop more slowly, are smaller, are evident first on the upper surface, rather than the lower as on NN plants, and show a much more distinct yellow halo than on NN plants. As both N and N′ factors are Mendelian dominants, and, as both affect the reaction of at least certain strains of tobacco mosaic on plants containing them, it was of interest to compare the reaction of the plantago and tobacco-mosaic viruses on heterozygous NN′ plants. Tests were first made on $F_2$ Burley 83–6 derived by 4 backcrosses of McMurtrey 8 N′N′ (Greenville, Tennessee, Tobacco Experiment Station) on an N burley. On field-size blooming NN′ plants of this hybrid in the greenhouse the tobacco-mosaic viruses, including those causing the necrotic reaction on N′N′ plants, all caused the usual necrotic spots. The plantago virus produced no reaction or such a weak reaction on these well-developed leaves as to be hardly noticeable. $F_3$ plants of 83–6, segregating for N and N′, inoculated with the plantago virus soon after transplanting to thumb pots, all developed necrotic spots and could have been classified as N. When the inoculated leaves were removed and the plants were inoculated with tobacco mosaic virus, they could be separated into those containing N and

those not containing it; because of necrosis in N plants and mottle in N′. Further tests were made on an $F_1$ hybrid of Pepper burley N′N′ and Ky. 48–7 NN. When these plants were growing rapidly in 4-inch pots, one half of a leaf was inoculated with tobacco mosaic and the other half with plantago virus. The large necrotic spots produced by the tobacco virus and the small spots of the plantago virus are shown in figure 2, B. Thus, in two hybrids the presence of the N′ factor has had no influence on the N reaction of the plant to the tobacco mosaic virus, but the N′ factor appears to dominate completely the reaction of the plant when inoculated with the plantago virus. In this respect the plantago virus differs from the strains of tobacco-mosaic virus that cause a necrotic reaction on N′N′ tobacco plants. On NN′ burley these tobacco-mosaic viruses cause the normal reaction of the tobacco-mosaic viruses on N plants.

### TOBACCO-MOSAIC VIRUS STRAINS TRANSFERRED TO PLANTAGO

Attempts to transfer 14 distinct field strains of tobacco-mosaic virus from tobacco to *Plantago major* in the late fall resulted in 12 instances in the development of local ill-defined to well-defined necrotic spots, sometimes surrounded by concentric necrotic rings, and, in 2 instances, of no local lesions. Fourteen transfers from new leaves of inoculated *Plantago major* plants to N burley after 44 days produced only 1 necrotic spot. From the 2 inoculated plantago leaves without symptoms, 63 spots developed from one and 54 from the other. From the 12 inoculated leaves with necrotic patterns, spots ranged from 19 to 530, with an average of 311. A disk from unrubbed leaves of plantago plants inoculated with the plantago virus developed 400 spots; another disk 190 spots. Further attempts in the spring to transfer tobacco-mosaic virus strains to *Plantago major* resulted in slowly developing chlorotic spots surrounded by chlorotic rings (Fig. 1, D). In no case has it been clearly demonstrated that the tobacco-mosaic virus became systemic in *Plantago major*. It is evident that the strains of tobacco-mosaic virus tested multiply in plantago, as Holmes found, but appear to be nearly completely localized.

### EFFECT OF PLANTAGO VIRUS ON TOMATO

As previously mentioned, the plantago virus studied in 1930 produced a faint mottle and some necrotic streaking in June Pink tomato. In the present studies, 7 Marglobe tomato plants, growing vigorously in a ground bench, were each inoculated, February 9, 1942, on three tip leaflets of one leaf about half way up the plant when the plants were about 27 inches tall. Other plants were inoculated in the same way with strains of tobacco mosaic that caused necrosis on N′N′ burley. The tobacco mosaics each caused systemic mosaic in tomatoes, but none of the plants inoculated with the plantago virus developed any symptoms whatever. Tests for the presence of virus in inoculated leaves and in growing points were made, March 9, 1942, on large 83–6 burley plants (N′N) before it was realized that they reacted only weakly to the plantago virus. The results were all negative from tomatoes inoculated

with plantago virus, but were positive with those inoculated with tobacco mosaics. Later, 16 Marglobe plants, 3 inches tall, were thoroughly inoculated on two leaves with one of 3 strains of plantago virus. All developed systemic mottling and 12 developed some streak. In April 3-inch June Pink plants, just becoming established in 4-inch pots, were inoculated. Three lots of 4 plants each were each inoculated with a different collection of the plantago virus. Two plants of each lot were inoculated on all leaflets of the first 2 leaves and the other 2 were inoculated on the tip half of 3 leaflets of 1 leaf. No local lesions developed, but soon systemic mottling occurred in the heavily inoculated plants, accompanied by mild necrotic streaking of the stalks and midveins and necrotic spots or rings in invaded leaves. As growth continued, symptoms were limited to isolated chlorotic spots in the new leaves that caused some distortion and prominent mottling. Of the lightly inoculated plants, only 2 became systemically invaded 3 weeks or more after inoculation. When the above test was repeated on 8-inch June Pink tomato plants in 6-inch pots, infection developed, after 35 days, only in the heavily inoculated plants.

Later, 4 Marglobe plants 20 to 27 inches tall were inoculated on 3 leaflet tips of 1 leaf or on all leaflets of 3 leaves about two-thirds up the plant. None of these plants developed systemic infection. In tests of the leaf tissue, using 3 0.6-cm. disks from actually inoculated tissue of each plant, only 5 of 11 trials produced necrotic spots on a necrotic-spotting NN burley.

The results of these tests suggest that tomato is resistant to the plantago virus, but, if young, vigorous plants are heavily inoculated, the virus will become systemic. As the leaves age they appear to become more resistant to both local and long-distance spread of the virus. The resistance of the tomato to the plantago virus is not very different from that of mosaic-resistant Ambalema burley hybrid plants to the tobacco-mosaic virus.

## DISCUSSION

The widespread distribution of a virus in plantago, which is so closely similar to the tobacco-mosaic virus that Holmes claimed "it was a strain of tobacco-mosaic virus (*Marmor tabaci* H.)," is of interest from the standpoint of the origin of a virus of a cultivated crop, such as tobacco. There seems to be no question that the plantago virus and the numerous field tobacco-mosaic virus strains are closely related. But to claim that plantago virus is a strain of the tobacco-mosaic virus implies that the changes in the virus have been from the tobacco-mosaic virus to the plantago virus, rather than in the opposite direction. On the contrary, it is not unreasonable to assume that the plantago virus antedates the allopolyploid species *Nicotiana tabacum* and cultivation of tobacco by the Indians, and, therefore, antedates the tobacco-mosaic virus itself. While there are species of *Nicotiana* that are susceptible to tobacco mosaic, it is doubtful whether in nature the virus maintains itself in these annual species. But there would be no difficulty in the virus maintaining itself indefinitely in a perennial species such as *Plan-*

*tago major* and attaining a very wide geographic distribution in the absence of any effective insect vector. Any mechanical means of distribution, such as the hoofs of animals tramping over diseased and then healthy plants, should be sufficient to bring about widespread distribution of a virus that withstands drying and is as easily transmitted as the plantago virus.

With the development of tobacco and its cultivation by the aborigines, the virus could readily have been transferred from plantago to tobacco, as illustrated on the Bell farm. If the variety of tobacco happened to be of the n′n′ genetic make-up, then a mottle disease would result which could be passed on indefinitely in cultivated tobacco grown by men who handled tobacco from the previous infected crop. Holmes has shown that there is a slight tendency for the plantago virus to develop substrains and in the present study a collection made in Connecticut developed the burning type of necrosis much more slowly in Ky. 16 than the local strains. Therefore it may be assumed that the virus is variable but confined, in its variability, to the limitations placed upon it by the genus *Plantago*.

Man, in handling tobacco plants with fingers contaminated by viruliferous dry tobacco, gives a means by which mutant strains can be isolated and then perpetuated. Blood (1) has demonstrated the ability of the tobacco mosaic virus to mutate sufficiently so that it could cause mosaic mottle in datura plants that previously showed the necrotic reaction. When the mutant virus was transferred to other datura plants, it no longer caused necrosis but caused a mottle disease. Thus by mutation, strains of the plantago virus might arise capable of causing mottle in N′ plants, and gradually the numerous field strains of the common tobacco mosaic could have been evolved. On the other hand, considering the high degree of resistance of plantago to systemic infection by the tobacco-mosaic virus, it is unlikely that the plantago virus originated from the tobacco-mosaic virus and was then distributed over its wide range in a relatively pure form.

In studies of the common field strains of the tobacco-mosaic virus for the past 20 years, the writers have failed to find any evidence for a type strain, but have found a nearly limitless series of strains, such as would be expected from a mutating species given opportunity to perpetuate its mutant strains. Each of the strains appears limited in its distribution, but any one of them may be maintained as a laboratory strain. Holmes has named the plantago virus *Marmor tabaci* var. *plantaginis*, thus making it coordinate with the strains of the tobacco-mosaic virus that he has recognized with Latin varietal designations. Obviously, it is not coordinate with them because it is a virus with well-defined characters, is well established in nature over a wide geographic range, and is found on a distinct group of plants, whereas his tobacco mosaic varieties are ill-defined, are not established in nature, and are obviously mutant strains of a well-recognized virus. This would seem sufficient, according to the criteria proposed by Valleau (7, p. 821), to give the plantago virus a varietal designation in contrast with the numerous mutant strains of the tobacco-mosaic virus, which should be given only laboratory designations.

Even though the plantago virus proves to be the stem from which the tobacco-mosaic viruses have arisen, it would seem necessary, because of priority, to designate it a variety of the species *tabaci*. According to the system proposed by Valleau, the virus would be called *Musivum tabaci* var. *plantaginis* new comb.

SUMMARY

The plantago virus is common in *Plantago* sp. in the vicinity of Lexington, Kentucky. An outbreak of a necrotic virus disease of burley tobacco was found to be caused by the plantago virus in a variety that responded with the necrotic reaction. In some other varieties, the same virus causes a mottle disease. Both types of reaction were found in tobacco plants naturally infected.

In tobacco carrying the N factor from *Nicotiana glutinosa*, the plantago virus and the tobacco-mosaic virus cause similar necrotic reactions, but, in tobacco carrying the N′, as well as the N, factor, the plantago virus produces few minute necrotic spots, while the tobacco mosaic virus causes the usual necrotic reaction.

The suggestion is made that the plantago virus may have antedated the origin of *N. tabacum* and that the numerous strains of the tobacco-mosaic virus may have derived from the plantago virus by mutation.

LITERATURE CITED

1. BLOOD, H. L., and R. D. WATSON. A modification of the tobacco mosaic virus No. 1 occurring in *Datura meteloides*. Utah Acad. Sci., Arts and Letters 15: 15–19. 1938.
2. HOLMES, FRANCIS O. A distinctive strain of tobacco-mosaic virus from Plantago. Phytopath. 31: 1089–1098. 1941.
3. JOHNSON, E. M., and W. D. VALLEAU. The ring symptom of virus diseases in plants. Kentucky Agr. Exp. Stat. Bull. 361. (See page 256 and figure 12D.) 1935.
4. VALLEAU, W. D., and STEPHEN DIACHUN. Virus distribution in mosaic-resistant tobacco and its relation to pattern development in susceptible varieties. Jour. Agr. Res. [U.S.] 62: 241–247. 1941.
5. ————, and E. M. JOHNSON. Burning and non-burning strains of tobacco mosaic. Kentucky Agr. Exp. Stat. Bull. 361. 1935.
6. ————. Localization and resistance to tobacco mosaic, in *Nicotiana*. Kentucky Agr. Exp. Stat. Bull. 360. 1935.
7. ————. Classification and nomenclature of tobacco viruses. Phytopath. 30: 820–830. 1940.

# THE ZONATE LEAF SPOT, A NEW DISEASE OF SORGHUM

D. C. Bain and C. W. Edgerton

(Accepted for publication June 29, 1942)

In the fall of 1940, a leaf spot was noted on the leaves of several varieties of sweet sorghum (*Sorghum vulgare* Pers.) growing on the experiment station grounds of Louisiana State University at Baton Rouge. Entire leaves of most of the plants were covered with large and somewhat zonate spots, and in these spots were numerous black sclerotia-like bodies.

Diseased leaves were placed in moist chambers, while bits of others were surface-sterilized and planted in bean-pod agar. A fungus with filiform conidia, borne in a slimy matrix, was obtained so consistently that it seemed advisable to make inoculation tests. Results of the inoculations indicated that the fungus was definitely pathogenic.

Attempts to identify the fungus were, from the beginning, unsatisfactory. It resembled in many ways a fungus that Miura (5) described from Manchuria in 1921 under the name *Ramulispora andropogonis* Miura. Later, in 1932, following the ideas of Bubak (4), the name was changed to *Titaeospora andropogonis* (Miura) by Tai (6). The genus *Titaeospora,* however, is characterized by the presence of branched conidia. Although the conidia of the sorghum fungus were definitely not branched, there was a possibility that the branching of the spores was not a fixed character and occurred only under certain conditions. Because of the apparent similarity of other characters, the sorghum fungus was then tentatively considered the same as the Manchurian fungus (1, 2). This, however, was later proved incorrect (3). In the summer of 1941, a fungus, definitely determined as *Titaeospora andropogonis,* was collected on Johnson grass (*Sorghum halepense* (L.) Pers.). This fungus bore the typical branched spores, as described for *Titaeospora,* and was shown to be different from the sorghum fungus. It seemed clear, then, that the sorghum fungus was not only a new species but also did not fit satisfactorily in any of the commonly known genera of the *Fungi Imperfecti.*

In the fall of 1941, a culture of the fungus, stained paraffin sections, and fresh mounts of the fruiting body on the host were sent to C. L. Shear for identification. Shear's reply was as follows: "This is a very interesting organism, and I have spent considerable time in attempting to identify it but have been unable to find any description of either genus or species which agrees with it. It suggests a *Cercospora* somewhat but differs in having slimy spore masses, and very short conidiophores and a *Sporodochium*-like base unlike most *Cerospora* species. It seems to be nearer the *Tuberculariaceae.* We are tentatively calling it *Gloeocercospora heterospora* n. gen. & sp. I do not feel quite sure at present whether all of the various forms, shapes, and sizes of conidia are mere variations of one form or whether there are two forms, one shorter and slightly thicker than the other. The sclerotia formed in culture are also an interesting feature of the fungus."

Based on a study of conidia from single-spore cultures and from the host, it is evident that the differences in the conidia are only variations of one form. Since the term "heterospora" suggests more than one distinct type of conidium, and since the organism occurs on species of the genus *Sorghum,* the name *Gloeocercospora sorghi* is now being proposed for the fungus.

## SYMPTOMS OF THE DISEASE

The leaf spots produced by *Gloeocercospora,* when well developed, can usually be distinguished from other sorghum leaf spots. Initial lesions ap-

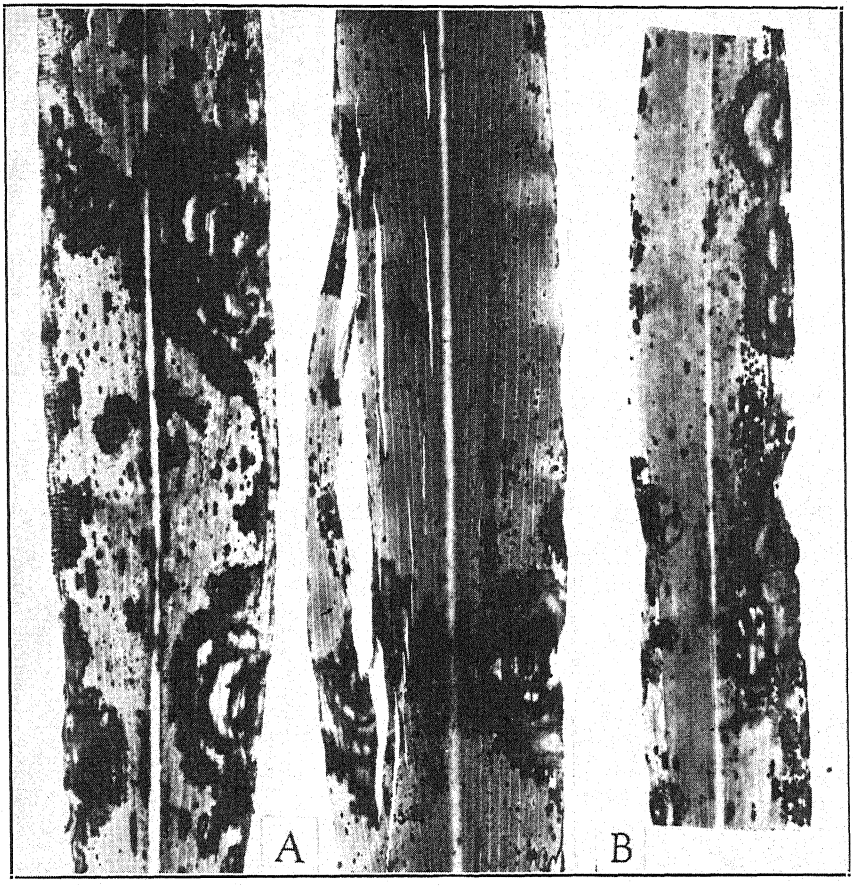



FIG. 1. Leaf spots produced by *Gloeocercospora sorghi* on leaves of sweet sorghum. A. Spots produced by natural infection. B. Spots produced by inoculation with pure culture.

pear as small, reddish or brownish, water-soaked spots that sometimes have a narrow, pale-green halo. Later, as the spots enlarge, they become a dark-red, except in certain varieties where they are light-brown, and become somewhat elongate and parallel to the veins. They finally form (possibly by coalescence) large, semi-circular, or irregular lesions several centimeters

in diameter (Fig. 1). A smaller lesion usually has a light-brown center, surrounded by a light- to dark-red border; but, frequently, in larger lesions there may be an alternation of dark and light zones. These leaf lesions may occur along the margins or towards the midrib, or they may cover the entire leaf when infection is heavy. Often the younger red lesions are so numerous as to form red irregular blotches. The zonate spots have been observed as early as the first part of June. Because of the characteristic type of spotting, the name "zonate leaf spot" is suggested for the disease.

A few weeks after infection occurs, minute spherical to lenticular sclerotia appear in the necrotic areas of infected leaves and sheaths. Leaves and sheaths are the only parts of the plant on which symptoms have been observed. The fungus, however, has been isolated from surface-sterilized seeds and glumes, which indicates that these structures also become infected.

## HOST RANGE AND DISTRIBUTION

The fungus was widely distributed in 1941 in southern Louisiana, being found on sorghum, Johnson grass, and Sudan grass (*Sorghum vulgare*, var. *sudanense* (Piper) Hitchcock). During the summer of 1941, typical zonate lesions were noted at Meeker, Louisiana, on one variety of sugarcane, C.P. 33/243. Leaves showing these lesions, when placed in a moist chamber, produced within a few days, conidia of *Gloeocercospora*. In 1942, the fungus was also collected on corn (*Zea mays* L.) at Baton Rouge.

The distribution of the fungus outside of Louisiana is indefinite. It was collected in 1941 on Johnson grass and sorghum at Poplarville, Mississippi. That it may have a wider distribution is indicated by a culture kindly sent by C. L. Lefebvre. This culture was isolated from Sudan grass from Arlington Farm, Virginia, in 1939. This isolate, except for slight cultural differences, proved to be quite similar to cultures isolated from sorghum in Louisiana. During 1942, specimens of the fungus on sorghum were received from G. F. Weber, Gainesville, Florida, and specimens of apparently the same fungus on seedlings of *Agrostis* were received from C. C. Wernham, State College, Pennsylvania.

## THE FUNGUS

*Gloeocercospora* sporulates on the host under field conditions but the large, well-defined fruiting bodies usually are inconspicuous. However, when infected material is placed in a moist chamber for 24 to 48 hours, an abundance of fruiting bodies appear in and surrounding necrotic areas.

From a study of prepared sections, it appears that the fruiting body is a sporodochium, and that this structure is found on the surface of the leaf above a stomate and arises from hyphae emerging from the stomate (Fig. 2, A). These hyphae branch and a sporodochial column is formed that, at maturity, is more or less definitely stalked. The branching conidiophores produce a bouquet-like structure (Fig. 2, B). The sporodochium apparently never originates within the tissue of the leaf and becomes

erumpent. There is no stromatic base above the leaf surface or in the substomatal cavity. The sporodochia are salmon colored and are easily visible to the naked eye. They occur either in dense clusters or sparsely in and around necrotic areas.

The conidiophores are so densely clustered that it is difficult to determine their length and width. In general, they appear to be short (5 to 10 μ), hyaline, and either simple or branched.

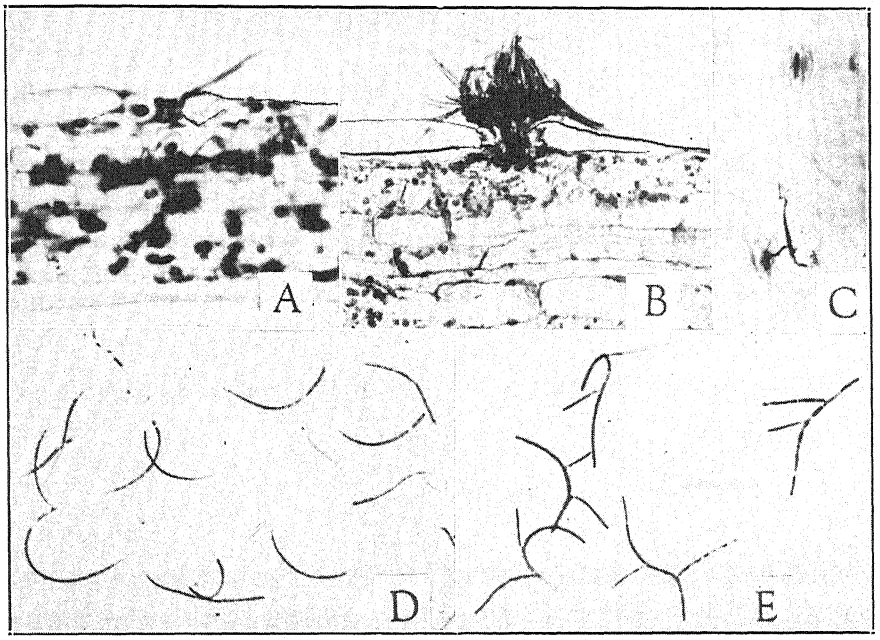



FIG. 2. A–D. *Gloeocercospora sorghi*. A. Young sporodochium, × 500. B. Mature sporodochium, × 320. C. Germ tube penetrating stomate, × 240. D. Spores, stained with cotton blue, × 320. E. Spores of *Titaeospora andropogonis*, stained with cotton blue, × 320.

The conidia (Fig. 2, D) are borne in a pinkish to salmon slimy matrix. They are either straight or curved, tapering somewhat from the base to the apex, few- to many-septate, hyaline, and elongate to filiform. The length varies from 20 to 195 μ and at the widest place they are slightly over 3 μ in width.

The black sclerotia develop within the tissues of the older leaf lesions. They occur at definite intervals and in lines parallel to the veins, which suggests that they form under the stomates. The host tissue, however, is so badly disintegrated by the time the sclerotia are fully developed that it is difficult to determine with certainty their relation to the stomates. In section, the sclerotia are round to elliptic. Each sclerotium has a central portion composed of pseudoparenchymatous tissue that is surrounded by a hard layer composed of thick-walled cells.

*Gloeocercospora* grows rapidly on ordinary culture media, often filling a

Petri dish in less than 10 days. It sporulates abundantly, especially on oatmeal agar. The optimum temperature for growth is in the neighborhood of 28° to 30° C.

The conidiophores in culture are hyaline, simple or branched, short, and septate. They have been observed to arise pleurogenously from hyphae as rather densely clustered short branches. Later, these clusters of conidiophores may become so dense as to form bouquet-like aggregates, resembling the sporodochia that are formed on leaves.

In culture the conidia, which develop in pink, bead-like, slimy masses, do not differ materially from those on leaf lesions. These masses often coalesce to form larger ones. Under optimum conditions, sporulation has occurred in 4 days. A conidium is attached somewhat on the side of the slightly swollen apex of the conidiophore (or its branch) and by its broadest end.

Sclerotia resembling closely those in the host develop on culture media.

The conidia germinate readily in water or culture media generally in less than 5 hours. Frequently, the different cells of the conidium produce germ tubes, as many as 6 having been observed coming from one spore.

In inoculation experiments it has been determined that the germ tubes of the germinating conidia enter the host through stomates. In the tests, potted sorghum plants in the greenhouse, plants growing in the field, and detached leaves in moist chambers were used. In one of the tests, the plants were kept under bell jars for 48 hours after being inoculated by spraying the leaves with a spore suspension. Beginning 6 hours after inoculation, material was killed and fixed in a 50 per cent solution of acetic acid in alcohol and cleared in a saturated solution of chloral hydrate. When ready for examination, leaf sections were stained with a dilute solution of cotton blue in lacto-phenol. The fungus took the stain, while the leaf tissue remained clear. Material killed 24 hours after inoculation was found most suitable for study.

In all the material examined, no appressoria were found, nor was there any evidence of penetration of the epidermis. Entrance of the germ tubes through stomates was observed in many instances (Fig. 2, C). In each one noted, penetration was effected before branching of the germ tube had occurred. Often, the germ tubes appeared slightly swollen at the point of contact with the stomatal aperture. It was noted also that germ tubes would frequently grow over and beyond nearby stomates without entering them.

In most of the experiments, brownish spots began to appear on the leaves about 24 hours after inoculation, apparently at about the time the germ tubes were entering the stomates.

## CLASSIFICATION OF THE FUNGUS

In the attempts to identify and classify the fungus, it has been necessary to compare it with organisms found in such genera as *Cylindrosporium*, *Titaeospora*, *Cercospora*, and *Cercosporella*. There is no evidence, however, that would warrant the placing of the sorghum fungus in any of these genera.

The fruiting body of *Gloeocerspora* is a sporodochium-like structure and, on this account, it is believed that the fungus should be placed in the *Tuberculareaceae*.

The genus *Cylindrosporium* is ordinarily placed in the *Melanconiales*, a group characterized by the presence of an acervulus, a structure that is innate in the matrix and finally becomes erumpent. A fruiting structure like that found in *Gloeocercospora*, which originates between the guard cells or slightly below and emerges through the stomate, cannot be considered an acervulus. For this reason, *Gloeocercospora* should not be placed in the genus *Cylindrosporium* or in the order *Melanconiales*. As the spore characters of *Gloeocercospora* resemble those of some of the species at present classified in the genus *Cylindrosporium*, it would be interesting to determine if some of the latter fungi may not have fruiting bodies similar to *Gloeocercospora*.

The fungus, *Titaeospora andropogonis*, is also at present placed in the *Melanconiales*. The fruiting body, however, is not a typical acervulus but a sporodochium-like structure, and the fungus probably should be placed in some other order. The stroma-like base of the sporodochium underneath the stomate and the branching of the spores (Fig. 2, E) are characters of *Titaeospora andropogonis*, which definitely distinguish it from *Gloeocercospora*.

*Gloeocercospora* differs from *Cercospora* and *Cercosporella* in that its fruiting body is definitely a sporodochium and the conidia are borne in a slimy matrix on short conidiophores.

## TECHNICAL DESCRIPTION

**Gloeocercospora** gen. nov.

Vegetative hyphae septate; fruiting body a sporodochium formed on the surface of the host above the stomatal opening and arising from hyphae that emerge through the stomate; conidiophores hyaline, septate, simple or branched, short; conidia hyaline, elongate to filiform, of variable length, the longer ones tapering, acrogenous, 1-multiseptate, straight or curved, borne in a slimy matrix.

**Gloeocercospora sorghi** sp. nov.

Vegetative hyphae septate, hyaline, branching; sporodochium between guard cells and above stomatal aperture; conidiophores hyaline, septate, simple or branched, short, 5 to 10 μ; conidia hyaline, elongate to filiform, of variable length, the longer ones tapering 20–195 × 1.4–3.2 μ, average 82.5 × 2.4 μ, borne in a slimy matrix, salmon-color in mass; sclerotia 0.1–0.2 mm. in diameter, lenticular to spherical, black, occurring inside the necrotic tissue of the host, abundant. Parasitic on leaves of *Sorghum halepense* (L.) Pers., varieties of *S. vulgare* Pers., and other grasses.

Type locality: Baton Rouge, Louisiana, U. S. A.

Type material including leaves, slides, and dried agar cultures of the fungus has been deposited in the herbarium of the Department of Botany, Louisiana State University and the mycological collections of the Bureau of Plant Industry, Washington, D. C.

DEPARTMENT OF BOTANY,
LOUISIANA STATE UNIVERSITY,
BATON ROUGE, LOUISIANA.

LITERATURE CITED

1. BAIN, DOUGLAS C. *Titaeospora andropogonis* (Miura) in Louisiana. Plant Dis. Rptr. 25: 142. 1941.
2. ————————. Preliminary studies of a sorghum leaf-spot in Louisiana. Proc. La. Acad. Sci. 6: 48. 1941.
3. ————————, and C. W. EDGERTON. Two-leaf spot diseases of sorghum and related grasses. (Abstract) Phytopath. 32: 1. 1942.
4. BUBAK, F. Pilze von verschiedene Standorten. Ann. Myc. 14: 345–349. 1916.
5. MIURA, M. Diseases of principal crops in Manchuria. Koshurei Agr. Exp. Sta. of the South Manchurian Railway Co. Report No. 11. Jan. 1921.
6. TAI, F. L. Notes on Chinese fungi: I. Nanking Jour. 2: 171–179. 1932.

# ANOTHER HYPHOMYCETOUS FUNGUS PARASITIC ON PYTHIUM OOSPORES

CHARLES DRECHSLER

(Accepted for publication July 22, 1942)

In an earlier paper (3) 2 hyphomycetous fungi, *Trinacrium subtile* Riess and *Dactylella spermatophaga* Drechsl., were reported as having been found subsisting parasitically on mature oospores formed by root-rotting oomycetes in isolation plate cultures prepared by planting sizable fragments of diseased vegetable tissue on maize-meal agar. From their morphological features and developmental traits these 2 fungi could be recognized as closely akin to the series of mucedinaceous forms most familiarly exemplified in *Arthrobotrys oligospora* Fres., the larger number of which, under natural conditions, habitually secure their nourishment by capturing nematodes, amoebae, and testaceous rhizopods.

More recently a third fungus of like biological habit and presumably also of like taxonomic kinship came to light in an old Petri-plate culture of *Pythium graminicolum* Subr., following the addition of a small quantity of leaf mold taken from woods near Haugen, Wis., in September 1939. Throughout a tract of approximately 500 square millimeters bordering the deposit of forest refuse, many thousands of oospores were found reduced to membranous remains. Day after day other oospores could be observed undergoing destruction along the margin of the slowly enlarging tract. The units of sexual apparatus about to be attacked arrested attention by the development of a distended crook-necked structure whose broadly rounded apex was closely appressed to the oogonial membrane; so that an appearance curiously suggestive of fertilization was presented, though true fertilization had everywhere been accomplished fully 2 months earlier. The crook-necked structure, while closely resembling the antheridia of various species of *Pythium,* including *P. graminicolum,* soon revealed its proper character by functioning as an appressorium in thrusting a process, often 1.5 to 2 μ wide, through the oogonial membrane and into the oospore wall. Within this wall the intruded process would sometimes gradually expand for about 2 hours and thus form a conspicuous bulbous enlargement 8 μ or 9 μ in transverse diameter (Fig. 1, A–D) before breaking into the chamber of the spore (Fig. 1, E) and extending throughout the protoplast a rather massive, somewhat branched haustorium (Fig. 1, F, G). Usually perforation of the oospore wall was accomplished with less pronounced inflation, the invading process, in most instances, becoming distended to a width of perhaps 4 μ or 5 μ in passing through the thick envelope (Fig. 1, H, I).

The protoplasmic content of the oospore usually revealed no change in its normal unitary organization (Fig. 1, A) until the invading fungus had almost penetrated the enveloping wall. At that stage the reserve globule often lost its smoothly circular contour, while, simultaneously, the refringent

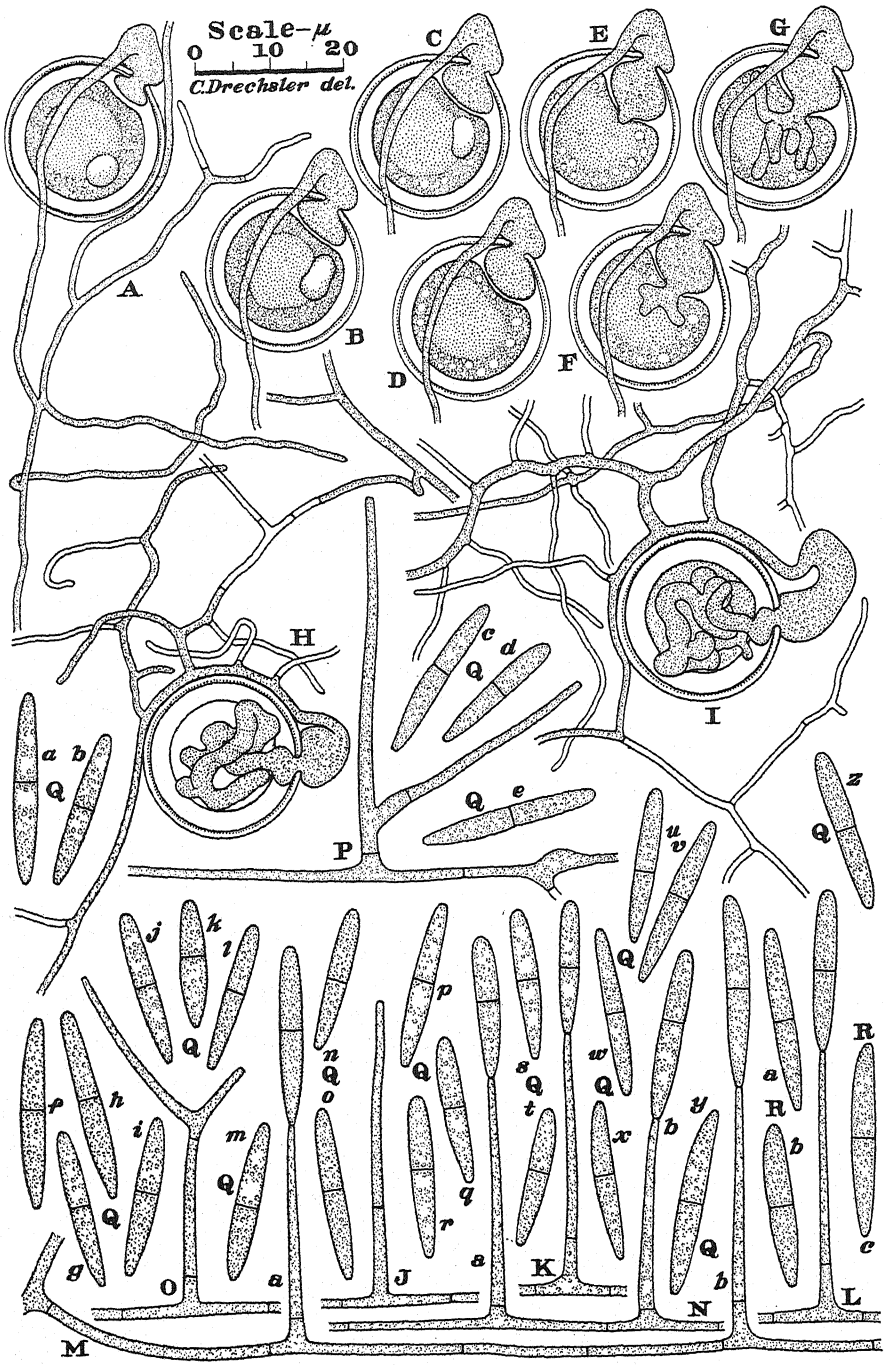



FIG. 1. *Trichothecium arrhenopum* drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A–G. Successive stages in the penetration and invasion of an oospore of *Pythium graminicolum* observed during 2½ hours. H, I. Portions of mycelium, each with an appressorium from which an oospore of *P. graminicolum* has been invaded. J–N. Portions of prostrate hyphae bearing one or two simple erect conidiophores. O, P. Branched conidiophores. Q, a–z; R, a–c. Random assortment of conidia, showing variations in size and shape.

body assumed an elongated (Fig. 1, B) or irregular outline. Soon afterwards the parietal granular layer became interspersed with numerous small vacuoles, and merged more and more with the misshapen reserve globule (Fig. 1, C, D). By the time the invading fungus had irrupted into the chamber of the oospore the refringent body often could no longer be clearly distinguished (Fig. 1, E–G). Gradually the disorganized mass of protoplasm became less dense in texture as the substances composing it were absorbed by the haustorium. The oospore wall became noticeably swollen and evidently underwent some softening (Fig. 1, H, I), though it was not apparent that the softened materials were assimilated. Eventually, after the granular residues of the host had disappeared, the protoplasmic contents of the haustorium itself were withdrawn into the external mycelium of the parasite.

This mycelium consisted of delicate hyphae giving off branches rather promiscuously in all directions (Fig. 1, A, H, I). Many of the finer ramifications were appreciably narrower than 1 μ, while the axial filaments from which they arose often did not exceed 1.5 μ in width. The delicate vegetative habit appeared to represent an adaptation whereby the parasite is enabled, with obvious economy, to seek out oospores in scattered positions. When an oogonium containing an oospore was encountered, the successful exploratory branch enveloped the oogonial membrane very tightly about half way around its circumference (Fig. 1, H, I) before giving rise terminally to the massive crook-necked appressorium already described. Unsuccessful exploratory branches, on the other hand, were soon evacuated through retraction of their protoplasm into the parent filaments. Septa could not be recognized within any portion of the exploratory mycelium that remained full of protoplasm, though partitions were readily visible within empty portions of hyphae, as well as at the boundaries of living portions. Perhaps intercellular septa, as distinguished from retaining walls, were omitted here in order to promote freer movement of protoplasm, and thus to facilitate the ready production and extension of exploratory branches. However, it is also possible that intercellular septa were present, but, owing to the small dimensions of the filaments, were indiscernible to ordinary microscopical inspection. The apparent continuity of its living portions made the submerged parasitic mycelium resemble in some degree the mycelium of a delicate phycomycete.

That the parasite is, nevertheless, not a phycomycete was evident from the indubitably septate condition of some coarser, more straightforward, and only sparingly branched hyphae formed on or near the surface of the agar medium (Fig. 1, J–P). These hyphae were concerned more directly with the asexual reproduction of the fungus than with its vegetative development, since they gave rise at moderate intervals to erect, slender, colorless, frequently uniseptate, aerial conidiophores (Fig. 1, J–L; M, a, b; N, a, b), which usually concluded their development by bearing a single colorless elongated conidium. Sometimes, to be sure, a conidiophore, after declining to the substratum, extended its usefulness by sending up a secondary

conidiophore as a lateral branch (Fig. 1, O, P); the secondary conidiophore, after bearing its single conidium, then occasionally falling over in turn and giving rise to a tertiary fertile branch. With respect to such protracted development, the fungus showed similarity not only to the oospore-destroying *Dactylella spermatophaga,* but also to many of the more robust members of the predaceous series of hyphomycetes subsisting habitually through capture of animals.

The conidia (Fig. 1, Q, a–z; R, a–c), whether produced on simple conidiophores or on branches arising therefrom, were only about two-thirds as wide and less than half as long as those of *Dactylella spermatophaga,* from which they differed further in being divided regularly by a single cross wall rather than by 3 cross walls. Their meager septation, blunt ends, and lack of pronounced curvature, removed them from any close resemblance to the macroconidia of *Fusarium,* though some general similarity to the much less distinctive microconidia produced by members of that genus was evident. Taxonomic disposition of the fungus is, therefore, not disturbed by such perplexities as are attached to the curiously ambiguous conidial morphology of *D. spermatophaga.*

The character of its asexual reproductive apparatus clearly refers the fungus to *Trichothecium* Link in the more restricted sense in which this genus was adopted by Saccardo (8, v. 4, p. 178). *Trichothecium,* throughout the *Sylloge Fungorum,* is held distinct from *Cephalothecium* Corda,—the former being made to include species that bear uniseptate, hyaline or light-colored conidia singly on simple erect conidiophores, while the latter is reserved for species bearing such conidia plurally in more or less capitate arrangement on conidiophores likewise erect, simple and septate. The distinction thus drawn has fallen somewhat into disfavor from a belief that *T. roseum* Link and *C. roseum* Corda, on which, respectively, the 2 genera would seem to have been founded, and by which, certainly, they have long been most familiarly exemplified, represent one and the same fungus. The fungus that presumably is in question here, best known to students of plant diseases from its causal connection with pink rot of apples, may, indeed, be found bearing solitary spores on conidiophores just at the beginning of their reproductiveness, whereas its older conidiophores are commonly found supporting a number of spores in a contorted spicate cluster having the general appearance of a head. Owing to the formation of the conidia one after another, in basipetal order, the clustered condition is necessarily preceded for a brief period in the development of the individual conidiophore by a condition that could be interpreted as expressive of a solitary sporulating habit; though the usual prevalence of a clustered arrangement wherever the fungus is present in quantity should remove virtually all occasion for such error.

The earlier definitions of *Trichothecium* were flagrantly wanting in morphological detail. Link failed to specify any particular manner of spore formation both in his first (5, p. 18) and in his second diagnosis (6 p. 28)

of the genus. Nor is any particular manner of spore formation mentioned in his diagnosis of *T. roseum* (6, p. 28); and the single relevant figure given by him (5, Tab. 1, 27) merely shows detached uniseptate spores scattered promiscuously among a number of septate hyphae. The usage that in early times grew up with regard to the species would seem to have been based more on considerations of color, habitat, and distribution than on considerations of morphology. Confusion of more than ordinary seriousness arose. As late as 1912 Grove (4) held that *T. roseum, T. candidum* Wallr., *T. obovatum* (Berk.) Sacc., *C. roseum, C. candidum* Bon., *Arthrobotrys superba* Corda, *A. oligospora,* and *A. rosea* Mass. might represent varying states of one species.

The citation of *Arthrobotrys superba* and of *A. oligospora,* with which *A. rosea* appears identical, among the dubious species of *Trichothecium* and *Cephalothecium* was without much justification, since the distinction between the truly capitate sporulation of *A. oligospora* and the successive, basipetal production of conidia in the pink-rot fungus had been repeatedly pointed out during the preceding half-century. This difference in manner of cluster development seems, indeed, of far greater taxonomic significance than the difference between production of single capitate clusters and successive capitate clusters; wherefore, some years ago, in describing as new 2 nematode-capturing species that give rise only to single capitate clusters, I ventured to assign them under the names *A. musiformis* and *A. dactyloides,* to *Arthrobotrys* (3) rather than to *Cephalothecium,* as would have been required in strict accordance with Saccardian usage. Through such extension of *Arthrobotrys* at the expense of *Cephalothecium,* the latter genus, of questionable validity because of its erection subsequent to *Trichothecium,* is conveniently excluded from the predaceous series of hyphomycetes. Of the 7 species compiled under *Cephalothecium* in the *Sylloge Fungorum, C. macrosporum* Speg. with fertile hyphae "apice minute abrupteque nodulosо-sterigmatophoris" (8, v. 10, p. 549), and *C. microsporum* Eichelb. with conidiophores "apice inflatis et minute verruculosis" (8, v. 22, p. 1305) might perhaps belong in the predaceous series. On the other hand, although Zopf's figure of *C. roseum* (9, p. 309, fig. 26, IV) shows uniseptate conidia borne in pronouncedly capitate arrangement on an erect conidiophore, the large, subspherical, terminal, *Oedocephalum*-like enlargement to which the conidia are attached seems scarcely less alien to *Arthrobotrys* than to *C. roseum* as originally described and figured by Corda (1, Tab. X, fig. 62).

The hyphomycetous forms intimately akin to *Arthrobotrys oligospora,* but producing their uniseptate conidia singly, offer no prominent morphological feature whereby they can be separated readily from the forms that while likewise producing solitary uniseptate conidia on erect sporophores are related to *Trichothecium roseum.* Under natural conditions of development a heavy, somewhat crustose, and rather bright-colored turf of sporiferous hyphae is likely to denote affinity with the pink-rot fungus, while scant development of colorless or only faintly tinged sporiferous hyphae would

more probably betoken affinity in the predaceous series,—the abundant display of conidial apparatus in the former instance being referable to direct utilization of the substratum as food supply, whereas the meager display in the latter instance derives from utilization only of minute animals or fungal structures associated with the substratum. Because of its very delicate aerial development *T. griseum* Speg. (8, v. 4, p. 180), for example, may be suspected of belonging in the predaceous series. The similarly very delicate *T. inaequale* (8, v. 18, p. 539) described by Massee and Salmon (7) from horse dung and rabbit dung in England, almost certainly is a member of this series, and very probably, in view of its close resemblance to the nematode-capturing fungus I described as *T. polybrochum* (2), habitually subsists on eelworms. As the fungus found parasitizing oospores of *Pythium graminicolum* gives rise to conidial apparatus in quantity so small as to be wholly invisible to the naked eye, its presence would ordinarily escape detection on opaque natural substrata. At all events, it cannot be identified with any of the 18 species compiled under *Trichothecium* in the *Sylloge Fungorum,* and has apparently not been described hitherto. It is, accordingly, presented as a new species under a specific name meaning "masculine-looking," which is intended to be descriptive of its antheridium-like appressoria.

**Trichothecium arrhenopum** sp. nov.

Mycelium bonam partem valde ramosum, hyphis incoloratis, certe ex parte septatis, plerumque 0.6–2 μ crassis, in oogonium Pythii maturum incasis id partim arte circumplectentibus denique appressorium ei late applicantibus; appressorio plerumque 5–8 μ crasso, 8–18 μ longo, ad instar antheridii curvato, membranas oogonii oosporaeque perforante denique ramos assumentes 1.5–3 μ crassos intrudente. Hyphae fertiles erectae, incoloratae, pauce septatae, plerumque 25–50 μ altae, basi 1.7–2.5 μ crassae, apice 0.7–1.5 μ crassae; conidiis solitariis, incoloratis, clavulatis vel elongato-ellipticis, basi obtusis, sursum rotundatis, medio septatis, plerumque 17–25 μ (saepe circa 21.4 μ) longis, 2.6–3.7 μ (saepe circa 3.1 μ) crassis.

Habitat in humo silvestri prope Haugen, Wisconsin.

Mycelium in large part abundantly branched, and in part, at least, septate; the vegetative hyphae colorless, mostly 0.6 to 2 μ wide, on encountering a mature *Pythium* oogonium enveloping it very closely along approximately one-half of its circumference and then producing an appressorium in broad contact with it; appressorium mostly 5 to 8 μ wide, 8 to 18 μ long, curved or crook-necked after the manner of some antheridia, after penetrating the walls of both oogonium and oospore intruding a haustorium with branches 1.5 to 3 μ wide to appropriate the protoplasmic contents. Conidiophores erect, colorless, usually with a single septum near the base, measuring mostly 25 to 50 μ (average 34.4 μ) in height, 1.7 to 2.5 μ (average 2.2 μ) in basal width, and 0.7 to 1.5 μ (average 0.9 μ) in apical width. Conidia solitary, colorless, somewhat clavate or of elongate-elliptical outline, blunt at the base, rounded at the tip, mostly 17 to 25 μ (average 21.4 μ) long, 2.6 to 3.7 μ (average 3.1 μ) wide, divided by a median septum, the position of which is sometimes marked by a slight constriction.

Occurring in leaf mold near Haugen, Wis.

## SUMMARY

A delicate mucedinaceous fungus from Wisconsin leaf mold was found energetically parasitizing oospores of *Pythium graminicolum* in an agar culture. It is described as a new species under the name *Trichothecium arrhenopum.* Like *Trinacrium subtile* and *Dactytella spermatophaga,* which have previously been made known as oospore parasites, it seems closely related to the series of predaceous hyphomycetes exemplified in *Arthrobotrys*

*oligospora* rather than to the familiar *T. roseum*. It penetrates the oogonial envelope and the oospore wall by means of a massive crook-necked appressorium having a curious resemblance to the antheridia of many species of *Pythium*.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND.

LITERATURE CITED

1. CORDA, A. C. J. Icones fungorum hucusque cognitorum. Tomus II. (Pragae.) 1838.
2. DRECHSLER, C. Some hyphomycetes that prey on free-living terricolous nematodes. Mycologia 29: 447–552. 1937.
3. ———. Two hyphomycetes parasitic on oospores of root-rotting oomycetes. Phytopath. 28: 81–103. 1938.
4. GROVE, W. B. New or noteworthy fungi: Part IV. Jour. Bot. 50: 9–18, 44–55. 1912.
5. LINK, H. F. Observationes in ordines plantarum naturales. Dissertatio I, complectens anandrarum ordines epiphytas, mucedines, gastromycos et fungos. Magaz. Ges. naturf. Freunde Berlin 3: 3–42. 1809.
6. ———. Species Hyphomycetum et Gymnomycetum. *In* Linnaei Spec. Plant. ed. 4, cura Willdenow, VI; Pars I: Hyphomycetes. 162 pp. G. C. Nauk (Berolini). 1824.
7. MASSEE, G., and E. S. SALMON. Researches on coprophilous fungi. II. Ann. Bot. 16: 57–93. 1902.
8. SACCARDO, P. A. Sylloge fungorum. . . . 25 v. Patavii, Abellini. 1882–1931.
9. ZOPF, W. Die Pilze. *In* Schenk, A. Handbuch der Botanik 4: 271–755. Edward Trewendt (Breslau). 1890.

# SEEDLING BLIGHT AND ROOT ROT OF GRASSES IN MINNESOTA[1]

E. A. ANDREWS

(Accepted for publication July 12, 1942)

There have been numerous complaints of failure to obtain stands of various species of grasses, even when seeds are sown in apparently favorable seed beds. Furthermore, 1-year-old plants have been observed injured by root rot in nurseries, the damage being especially severe on the Forage strain of crested wheatgrass.

Because of the difficulties encountered in obtaining and maintaining stands of grasses, work was begun in the fall of 1939 to determine the cause. Seed of the following grasses were surface sterilized for 3 minutes in mercuric chloride and plated out on acid potato-dextrose agar: *Bouteloua gracilis* (H. B. K.) Lag., *B. curtipendula* (Michx.) Turr., *Agropyron cristatum* (L.) Beauv., *A. pauciflorum* (Schwein.) Hitchc., *A. smithii* Rydb., *Andropogon furcatus* Muhl., *A. scoparius* Michx., *Bromus inermis* Leyss., *Buchloë dactyloides* (Nutt.) Engelm., *Elymus canadensis* L., *Panicum virgatum* L., *Poa ampla* Merr., and *Sorghastrum nutans* (L.) Nash. Many fungi, including *Helminthosporium, Alternaria, Fusarium, Cladosporium,* and *Penicillium* spp., were obtained from a total of 1,500 seeds. Bacteria, also, were commonly isolated.

Surface-sterilized and nonsterilized seed of the above species of grasses also were planted in steamed and nonsteamed sand. No significant differences were observed in germination capacity, time of germination, or in seedling vigor. Therefore, work with the organisms isolated from seed was discontinued and attempts were made to isolate organisms from the roots of grass seedlings grown in soil from different sources.

Seed of *Agropyron smithii, Andropogon scoparius, Bouteloua gracilis, B. curtipendula, Bromus inermis, Poa ampla,* and *Sorghastrum nutans* were planted in the greenhouse in soils abtained from Brookings, South Dakota, and Waseca and St. Paul, Minnesota. Organisms were isolated from the roots of the resulting plants by surface-sterilizing tissue with mercuric chloride and then plating out on acid potato-dextrose agar. The isolates obtained were then tested for pathogenicity in a preliminary way by placing them in a plate of water agar in which seedlings of bromegrass or crested wheatgrass were growing. If the roots of the seedling turned brown or died, the isolate was considered pathogenic. Table 1 lists the organisms isolated from roots in each of the soils and the pathogenic effects of each.

[1] Paper No. 2010, Scientific Journal Series, Minnesota Agricultural Experiment Station.

Assistance in the preparation of these materials was furnished by the personnel of the Works Projects Administration, Official Project No. 165–1–71–124, sponsored by the University of Minnesota.

The writer wishes to acknowledge his indebtedness to Dr. E. C. Stakman, Dr. Ian W. Tervet, Dr. J. J. Christensen, and other members of the Division of Plant Pathology and Botany, University of Minnesota, for helpful suggestions and guidance throughout the experiments and the preparation of the manuscript.

TABLE 1.—*Soil borne organisms isolated from the roots of grass seedlings grown in soils from three different sources*

| Organisms isolated | Number of times isolated from soils[a] | | |
|---|---|---|---|
| | Brookings, S.D. | Waseca, Minn. | St. Paul, Minn. |
| *Fusarium* sp. | 4 p | 3 p<br>1 s | 1 p<br>3 s |
| *Helminthosporium sativum* | – | – | 3 p |
| *Alternaria* sp. | – | – | 1 p<br>1 s |
| *Stilbaceae* | – | 1 p<br>1 s | – |
| Unknown fungi | 1 p | 3 s | 2 p<br>3 s |

[a] (p) indicates pathogenic, (s) indicates saprophytic.

Additional isolations were made from naturally infected soil obtained from the agronomy plots at University Farm, St. Paul, Minn., in which 1-year-old crested wheatgrass plants were severely damaged by root rot. For purposes of isolation, seeds of the Forage and Fairway strains of crested wheatgrass were planted in the greenhouse in the soil on which the diseased crested wheatgrass plants had been growing. Some of the root tissue of the resulting seedlings was surface-sterilized with mercuric chloride before being plated out, while some was washed and soaked repeatedly in sterile distilled water before being plated out on water agar and potato-dextrose agar. The following organisms were obtained: 14 isolates of *Pythium graminicolum*[2] Subramaniam, 8 isolates of *Fusarium* spp., 2 isolates of *Rhizoctonia solani* Kühn, 2 isolates of *Helminthosporium sativum* P.K. and B., 1 isolate of *Alternaria* sp., and 4 isolates of *Trichoderma* sp., along with 11 unknown fungi. Most of the cultures of *Pythium*[2] were obtained by the second method of isolation.

### GREENHOUSE PATHOGENICITY TEST

The pathogenicity of some of the organisms obtained from the Waseca and St. Paul soils, as well as some from the agronomy plot soil, was tested in the greenhouse and the field. In the greenhouse *Helminthosporium sativum* isolated from the roots of *Bouteloua gracilis* grown in St. Paul soil, *Fusarium* sp. isolated from the roots of *Sorghastrum nutans* grown in Waseca soil, and *Rhizoctonia solani* and *Pythium graminicolum* isolated from the roots of crested wheatgrass grown in the agronomy plots at University Farm were introduced into the soil by distributing bits of mycelium on the surface of soil in 4-inch pots at the time of planting. This method of inoculation was used in all experiments except where otherwise stated.

[2] The cultures of *Pythium graminicolum* isolated from infected soil in the agronomy plots of University Farm, St. Paul, Minn., produce oogonia readily on cornmeal and potato-dextrose agars. When grown in potato-dextrose broth for 4 days and then transferred to sterile distilled water, the mycelium produces typically lobed sporangia, vesicles, and zoospores within 48 to 60 hours. The granular protoplasm passes from the sporangium into a vesicle at the end of a slender tube where it differentiates into 50 to 60 swarmspores per vesicle.

Three hundred seeds each of bromegrass and crested wheatgrass were planted in the above soils; and one planting was made in steamed soil as a check.

TABLE 2.—*Survival of Bromus inermis and Agropyron cristatum in the greenhouse in steamed soil inoculated with four fungi*

| Organisms | Number of surviving plants of the two grasses[a] | |
|---|---|---|
| | *A. cristatum* | *B. inermis* |
| *Helminthosporium sativum* | 0 | 29 |
| *Rhizoctonia solani* | 61 | 73 |
| *Fusarium* sp. | 66 | 96 |
| *Pythium graminicolum* | 0 | 38 |
| None | 72 | 116 |

[a] One hundred and fifty seeds of each species of grass.

It appears that *Helminthosporium sativum* and *Pythium graminicolum* reduced the stands of both grasses about equally. Preemergence killing was the most conspicuous type of injury caused by both. Of the brome seedlings that survived in the inoculated soil only a few died after emergence.

Stakman (6), Christensen (2), and Dosdall (4) reported about 20 years ago that *Helminthosporium sativum* could cause seedling blight and root rot of grasses. The writer's results confirm theirs. Sprague and Atkinson (5), and Buchholtz (1) have recently reported seedling blight and root rot of grasses caused by *Pythium.*

Field tests were next made. *Helminthosporium sativum, Rhizoctonia solani, Pythium graminicolum,* and *Fusarium* sp., isolated from crested wheatgrass grown at University Farm, St. Paul, Minn., were used to inoculate crested wheatgrass. The fungi were grown on 70 cc. of a mixture of 95 per cent sand and 5 per cent cornmeal by volume in 8-oz. jars. One hundred seeds of the Fairway strain of crested wheatgrass were mixed with each culture; and the seeds were then planted with the inoculated culture medium in 2-foot rows, 36 hours after mixing. Five 2-foot rows were planted in each of the 3 replicates, making a total of 1500 seeds planted for each treatment. Noninoculated steamed sand and cornmeal mixture was used for the control plots. The experiment was planted on September 9, 1941, and the stand count was made on October 25.

Although the experimental set-up was not such as to permit statistical analysis, *Helminthosporium sativum* reduced stands so consistently and so greatly that the results appear significant. *Pythium graminicolum* and *Fusarium* sp. reduced stands also, but very much less than *H. sativum.* The isolate of *Rhizoctonia solani* used did not cause appreciable damage; in fact, the average stand was somewhat better than that in the check plots, but because of variation in replicates it is doubtful if the difference is significant. Table 3 gives the stand counts for each of the replicates and the total stand for each treatment as a percentage of the check.

TABLE 3.—*Stand counts of the Fairway strain of Agropyron cristatum grown in the field in noninoculated soil and in soil inoculated with 4 fungi*

| Organism | Stand count of replicates[a] | | | Total stand counts in per cent of check |
|---|---|---|---|---|
| | a | b | c | |
| *Helminthosporium sativum* | 6 | 4 | 11 | 6.3 |
| *Rhizoctonia solani* | 149 | 150 | 132 | 129.4 |
| *Fusarium* sp. | 103 | 63 | 34 | 60.2 |
| *Pythium graminicolum* | 60 | 71 | 46 | 53.2 |
| None | 109 | 66 | 158 | 100.0 |

[a] Five hundred seeds sown in each replicate.

In further greenhouse experiments 14 species of grasses were tested against one isolate of *Pythium graminicolum* isolated from the roots of crested wheatgrass growing at University Farm, St. Paul, Minn. The tests were made in steamed soil that had been inoculated with the isolate at the time of planting. Check plants were grown in noninoculated steamed soil. Two stand counts were taken, 19 days and 46 days, respectively, after planting. Table 4 gives the stand counts of plants growing in the steamed soil and the inoculated soil and shows the amount of post-emergence killing. There was severe preemergence killing of all grasses and many seedlings were killed after emergence.

TABLE 4.—*Stand counts of 14 species of grasses grown in noninoculated steamed soil and in steamed soil inoculated with Pythium graminicolum in the greenhouse*[a]

| Grass | Noninoculated soil | | Inoculated soil | |
|---|---|---|---|---|
| | Number of plants surviving | | Number of plants surviving after 19 days | Plants surviving after 46 days expressed in percentage of check |
| | After 19 days | After 46 days | | |
| *Agropyron cristatum* | 66 | 62 | 11 | 4.8 |
| *A. pauciflorum* | 46 | 40 | 28 | 42.5 |
| *A. smithii* | 45 | 50 | 16 | 32.0 |
| *Andropogon furcatus* | 21 | 15 | 3 | 6.8 |
| *A. scoparius* | 36 | 33 | 10 | 6.5 |
| *Bouteloua curtipendula* | 30 | 29 | 0 | 0.0 |
| *B. gracilis* | 31 | 27 | 0 | 0.0 |
| *Buchloë dactyloides* | 9 | 9 | 2 | 11.1 |
| *Elymus canadensis* | 14 | 14 | 1 | 7.1 |
| *E. junceus* | 48 | 46 | 7 | 8.7 |
| *Oryzopsis hymenoides* | 22 | 21 | 0 | 0.0 |
| *Panicum virgatum* | 62 | 44 | 3 | 0.0 |
| *Poa ampla* | 47 | 47 | 15 | 10.1 |
| *Stipa viridula* | 51 | 42 | 0 | 0.0 |

[a] Seventy-five seeds of each species planted.

## EFFECT OF SOIL TEMPERATURE ON THE PATHOGENICITY OF PYTHIUM GRAMINICOLUM

It was considered possible that soil temperature might influence the pathogenicity of *Pythium graminicolum*. Therefore, seedlings of *Agropyron*

*cristatum* and *Bromus inermis* were grown at 7 soil temperatures in steamed soil and in steamed soil inoculated with *P. graminicolum*. The experiment was conducted in temperature tanks (3), the seeds being sown in 4-inch pots. In half of each pot 25 seeds of *B. inermis* were planted and in the other half 25 seeds of *A. cristatum*. Three isolates of *P. graminicolum* were tested, 2 pots of each isolate and 2 pots of noninoculated steamed soil being maintained at each soil temperature.

In this experiment temperature had no appreciable effect on the pathogenicity of any of the isolates (Table 5). Although *Bromus inermis* appeared slightly more resistant to isolate 59 than *Agropyron cristatum*, the stands of both grasses were markedly reduced by all 3 isolates.

TABLE 5.—*Seedling survival of Bromus inermis and Agropyron cristatum at 7 temperatures in soil inoculated with 3 isolates of Pythium graminicolum*

| Soil temperature in degrees C. | Isolate and number of seedlings surviving 18 days after planting[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Bromus inermis* | | | | *Agropyron cristatum* | | | |
| | 27 | 50 | 59 | check | 27 | 50 | 59 | check |
| 12.3 | 1 | 1 | 2 | 39 | 0 | 0 | 0 | 25 |
| 17.0 | 0 | 3 | 3 | 28 | 0 | 0 | 0 | 20 |
| 18.0 | 0 | 2 | 3 | 33 | 0 | 0 | 0 | 19 |
| 24.3 | 0 | 0 | 7 | 37 | 0 | 0 | 0 | 16 |
| 25.6 | 0 | 0 | 1 | 36 | 0 | 0 | 0 | 15 |
| 28.3 | 2 | 0 | 2 | 34 | 0 | 0 | 0 | 19 |
| 31.5 | 3 | 1 | 5 | 34 | 0 | 0 | 1 | 21 |

[a] Fifty seeds of each species planted in each treatment.

## SUMMARY

*Helminthosporium, Alternaria, Fusarium, Cladosporium, Penicillium* spp., and many cultures of unknown fungi and bacteria were isolated from 1,500 seeds of 11 species of grasses.

Seed treatment with mercuric chloride did not produce significant differences in germination, emergence, or seedling vigor when the grasses were grown in steamed sand.

Preliminary pathogenicity tests on water agar, made with some fungi isolated from the roots of grasses grown in soils from Brookings, S. Dak., Waseca, Minn., and St. Paul, Minn., indicated that some organisms from each source were pathogenic.

*Helminthosporium sativum,* isolated from roots of *Bouteloua gracilis* grown in St. Paul soil and from the roots of *Agropyron cristatum* grown in soil obtained from the agronomy plots of University Farm at St. Paul, Minn., caused severe preemergence killing, reduction of root development, and stunting of *A. cristatum* and *Bromus inermis* in the greenhouse.

*Pythium graminicolum,* isolated from roots of *Agropyron cristatum* grown in soil obtained from the agronomy plots of University Farm, St. Paul, Minn., caused preemergence killing, reduction of root systems, and

stunting in all experiments. Post-emergence killing also was common in 4 of 14 species of grasses growing in soil inoculated with this organism.

The pathogenicity of *Pythium graminicolum* on *Bromus inermis* and *Agropyron cristatum* was similar at soil temperatures ranging from 12.3° to 31.5° C.

UNIVERSITY FARM, ST. PAUL,
MINNESOTA.

LITERATURE CITED

1. BUCHHOLTZ, W. F. Gross pathogenic effects of *Pythium graminicolum, P. debaryanum*, and *Helminthosporium sativum* on seedlings of crested wheat grass. (Abstract) Phytopath. 32: 2. 1942.
2. CHRISTENSEN, J. J. Studies on the parasitism of *Helminthosporium sativum*. Minn. Agr. Exp. Stat. Tech. Bull. 11. 1922.
3. ————. The relation of soil temperature and soil moisture to the development of head smut of sorghum. Phytopath. 16: 353–357. 1926.
4. DOSDALL, L. Factors influencing the pathogenicity of *Helminthosporium sativum*. Minn. Agr. Exp. Stat. Tech. Bull. 17. 1923.
5. SPRAGUE, R., and R. E. ATKINSON. Cross inoculations with *Pythium arrhenomanes* from cereals and grasses in the Northern Great Plains. (Abstract) Phytopath. 32: 17. 1942.
6. STAKMAN, L. J. A Helminthosporium disease of wheat and rye. Minn. Agr. Exp. Stat. Bull. 191. 1920.

# THE EFFECT OF PHOTOPERIODISM ON THE DEVELOPMENT OF BUNT IN TWO SPRING WHEATS

H. A. RODENHISER AND J. W. TAYLOR

(Accepted for publication July 9, 1942)

## INTRODUCTION

In a recent abstract (4) reference was made to the bunt reaction of Hope and Marquis wheats when grown at different day lengths under greenhouse conditions. In these experiments, Hope plants, exposed to light (1) continuously, (2) 10–11 hours daily, and (3) 8 hours daily, developed 64.1, 17.5, and 0.8 per cent smut, respectively. The corresponding percentages for Marquis were 32.7, 17.8, and 1.9. These results have a bearing on studies concerning the nature of resistance. Further tests, therefore, were made to determine the effectiveness of continuous light in changing the bunt reaction of a variety known to be resistant under a wide range of field conditions.

## METHODS AND EXPERIMENTAL RESULTS

Seed of the spring wheat variety Canus (C.I.[1] 11637) was inoculated with chlamydospores of *Tilletia levis* Kühn races L–1, L–2, and L–4 and of *T. tritici* (Bjerk.) Wint. races T–1, T–4, T–9, T–10, and T–12. Canus had been tested previously for its bunt reaction under a wide range of field conditions and found, when spring-sown, to be consistently resistant to all races used in this experiment (5). The inoculated seed was germinated and grown to a height of approximately 1 inch in soil at 10° C. and then transplanted to greenhouse benches at Arlington Experiment Farm, Arlington, Va. In 1940, one block of seedlings, containing duplicate rows of 72 plants per row, and in 1941 single rows with the same number of plants for each of the races listed in table 1, were subjected to continuous light, beginning 2 days after transplanting and continuing until noninfected plants were in the soft dough stage. Daylight was supplemented from 4 p.m. to 8 a.m. by 100-watt Mazda lamps suspended at a distance of approximately 24 inches above the plants. The light intensity at the top of the plants during this period was approximately 50 ft.-c. Electric fans were used to circulate the air around the artificially illuminated plants. This kept the night-air temperature within approximately + 3° C. of that in the control series. During the time of these tests, the period from sunrise to sunset averaged approximately 11 hours. A control series of Canus received no supplementary illumination. In 1941 comparable tests were made with the spring variety Ulka (C.I. 11478), which, under field conditions, is susceptible to all races used in this experiment, except T–10. In 1940, this variety was included only in the continuous light series.

It is clear from the results presented in table 1, that a high degree of resistance to each of the races was maintained when the plants were exposed

[1] C.I. refers to accession number of the Division of Cereal Crops and Diseases.

to an 11-hour light period under greenhouse conditions. However, exposure to continuous light effected a marked lowering of the resistance of Canus to certain races. The average percentages of heads smutted by races L–1, L–2, and L–4 in the 11-hour day series were 2, 4, and 3, respectively, while the corresponding percentages for the continuous light series were 42, 55, and 37. Similar results were obtained with *Tilletia tritici* race T–4. With this race an average of 4 per cent smut developed with the 11-hour day in contrast to 59 per cent in the series with continuous light. It is apparent also from the data in table 1 that the effect of continuous light in lowering the resistance of Canus is specific only for certain races. For example, with T–1, an average of 22 per cent bunt developed in the continuous-light series in contrast to the much higher percentages obtained with the races referred to above. Furthermore, the prolonged-light treatment was relatively ineffective in lowering the resistance of Canus to races T–9, T–10, and T–12 where average percentages of smut in the natural day and continuous light series were 1, 1, 0, and 4, 10, and 12, respectively.

In extensive field tests with Canus and Ulka, the latter variety was completely susceptible to all races except T–10 (5). With this exception, it was likewise susceptible in the present 11-hour day and continuous-light greenhouse experiments. It is noteworthy from the results in table 1, however, that the percentages of smut were slightly higher with all races under the latter conditions. Ulka, usually intermediate in its reaction to T–10 under field conditions, continued to be so in the 11-hour day greenhouse tests, but

TABLE 1.—*Reaction of Canus and Ulka to certain races of Tilletia levis and T. tritici when grown under two different day lengths in the greenhouse at Arlington, Va., in 1940 and 1941*

| Variety | Race of smut | Percentage smut[a] | | | | Average | |
|---|---|---|---|---|---|---|---|
| | | 1940 | | 1941 | | | |
| | | 11-hr. day (87) | 24-hr. day (59, 58) | 11-hr. day (83, 87) | 24-hr. day (47, 47) | 11-hr. day | 24-hr. day |
| Canus | L– 1 | 3 | 39 | 0 | 45 | 2 | 42 |
| | L– 2 | 6 | 36 | 1 | 74 | 4 | 55 |
| | L– 4 | 6 | 28 | 0 | 46 | 3 | 37 |
| | T– 1 | 1 | 16 | 1 | 27 | 1 | 22 |
| | T– 4 | 8 | 54 | 0 | 63 | 4 | 59 |
| | T– 9 | 1 | 3 | 0 | 5 | 1 | 4 |
| | T–10 | 1 | 11 | 0 | 9 | 1 | 10 |
| | T–12 | 0 | 12 | 0 | 12 | 0 | 12 |
| Ulka | L– 1 | ..... | 100 | 80 | 75 | 80 | 88 |
| | L– 2 | ..... | 98 | 69 | 73 | 69 | 86 |
| | L– 4 | ..... | 100 | 68 | 75 | 68 | 88 |
| | T– 1 | ..... | 92 | 71 | 79 | 71 | 86 |
| | T– 4 | ..... | 95 | 74 | 71 | 74 | 83 |
| | T– 9 | ..... | 75 | 64 | 83 | 64 | 90 |
| | T–10 | ..... | 25 | 16 | 56 | 16 | 41 |
| | T–12 | ..... | 98 | 68 | 77 | 68 | 88 |

[a] Figures in parentheses indicate number of days from emergence to heading for Canus (first) and Ulka (second).

the percentage infection was increased when the variety was grown under continuous light, the comparative infection percentages being 16 and 41, respectively.

In these experiments, differences were observed in the growth types of both infected and noninfected plants of Canus and Ulka in the two different light conditions. By comparison, the plants under continuous light were slightly less vigorous, produced fewer tillers, were approximately 3 inches shorter, and headed in a shorter period, than did those in the series without supplementary illumination. Data on the rapidity of growth, indicated by the relative number of days required for heading, are recorded in parentheses in table 1. Because of shortage of greenhouse space in 1940, Ulka was omitted in the control series, but in 1941, data were obtained on both Canus and Ulka. In 1941, Canus headed 36 days earlier under continuous light than in the 12-hour day and Ulka 40 days earlier. As noted in table 1, it was the plants grown under the 24-hour day that had the higher percentage of bunt. Thus it is evident that at least in these varieties certain races of bunt fungi, once established in the host, may keep pace with the growing point even when the culms are elongating at an exceptionally rapid rate.

The effect of illumination on the reaction of varieties to various smuts has been studied by other investigators. Reed (3) investigated its effect on the reaction of oats to *Ustilago avenae* (Pers.) Jens. and *U. levis* (Kell. and Sw.) Magn. Under artificial illumination, oats headed several days to 4 weeks in advance of the nonilluminated controls, but, contrary to some of the present results with wheat, oats gave no evidence of any change in either susceptibility or resistance of the varieties to the races used. Lasser (2) reported marked differences in the development of oat plants under various conditions of illumination, but no significant variation in the percentages of smut was recorded. Similar results were obtained in his tests with spring wheat varieties inoculated with *Tilletia tritici*. In preliminary tests, the writers have studied the effects of different day lengths on the development of *Ustilago tritici* (Pers.) Rost. in the soft red winter wheat variety Wabash (C.I. 11384). The number of days from seeding to heading was 81, 112, and 122 for continuous light, 11-hour-, and 8-hour-day periods, respectively. The percentages of smut infection for the corresponding periods were 67, 74, and 79, indicating a slight trend, but no very definite relation between rate of growth in the host and percentage of loose smut. It seems evident, therefore, that the effect of illumination in changing the host reaction to smuts is specific for certain hosts and races of the fungi involved.

## DISCUSSION AND CONCLUSIONS

It is clear from the results obtained that continuous light has a marked effect in lowering the resistance of Canus wheat to certain races of *Tilletia levis* and *T. tritici*. By controlling the light factor, changes in the general vigor of the host plants were effected. It is questionable, however, if any of these changes in vigor can be correlated with the breakdown in smut re-

sistance. The reaction of Canus to the races used in the present test has previously been studied under a wide range of field conditions. When spring-planted, the range in vigor of growth at different stations was even greater than that observed under the natural day and under continuous light in the greenhouse; yet, in all of the spring-planted field tests, the variety maintained a high degree of resistance. There is evidence also that the resistance of Canus to some of the races used in the present test can be altered without appreciable change in the growth habit of the host. Canus was inoculated, among other races, with L–1, L–2, L–4, T–4, T–9, and T–10 and fall-planted at Pendleton, Oregon, in 1937 and 1938 (5). Although there was no appreciable difference in the vigor of growth of the host in the two seasons, it was resistant in 1937 to all races, and, in 1938, intermediate in reaction. It is noteworthy that in these same field tests, Canus was more susceptible to T–9 and T–10 than it was in the greenhouse under continuous light, indicating that some other factors were responsible for the change in reaction.

In field tests, low percentages of bunt frequently have been obtained in what appeared to be rapidly growing plants. It is often assumed that under these conditions the bunt fungus grew more slowly than the wheat plant and failed to keep pace with the growing point of the shoots. It is evident from the present results, however, that these races of the bunt fungi kept pace with the host under exceedingly rapid growing conditions. Possibly the rate of growth of the fungus is conditioned by certain nutritional changes in the host some of which are brought about by prolonged exposure of the host plants to light (1).

## SUMMARY

Canus and Ulka wheats inoculated with 3 races of *Tilletia levis* and 5 of *T. tritici* were grown at natural-day and continuous-light conditions in the greenhouse at Arlington Farm, Virginia. Under natural-day conditions Canus was resistant to all races, while under continuous light a marked breakdown in resistance to certain races but not to others was observed. Ulka was completely susceptible to all but one race under both day-length conditions, and resistance to this one was lowered by the long-day treatment.

Differences in vigor of the host plants in the two light conditions were observed, but none of the changes could be definitely correlated with the breakdown in smut resistance.

The highest percentages of bunt were obtained in plants growing at an exceedingly rapid rate. It is suggested that the rate of growth of the bunt fungus may be conditioned by certain nutritional changes in the host that may have been brought about by prolonged exposure of the host plants to light.

BUREAU OF PLANT INDUSTRY STATION,
U. S. DEPARTMENT OF AGRICULTURE,
BELTSVILLE, MD.

LITERATURE CITED

1. HURD-KARRER, ANNIE M., and ALLAN D. DICKSON. Carbohydrate and nitrogen relations in wheat plants with reference to type of growth under different environmental conditions. Plant Physiology 9: 533–565. 1934.
2. LASSER, E. Der Einfluss von Licht unter Jarowisation auf den Befall von Weizen, Hafer und Gerste durch *Tilletia, Ustilago,* und *Helminthosporium.* Kühn Archiv. 44: 161–211. 1937.
3. REED, GEORGE M. Influence of the growth of the host on oat smut development. Proc. Amer. Philosoph. Soc. 79: 303–326. 1938.
4. RODENHISER, H. A., and J. W. TAYLOR. Studies on environmental factors affecting infection and the development of bunt in wheat. (Abstract) Phytopath. 30: 20. 1940.
5. ————, and C. S. HOLTON. Variability in reaction of wheat differential varieties to physiologic races of *Tilletia levis* and *T. tritici.* Phytopath. 32: 158–165. 1942.

# THE ROLE OF PACKING METHODS IN THE INCREASE OF ANTHRACNOSE OF HONEYDEW MELON FRUITS[1]

W. A. KREUTZER AND D. P. GLICK[2]

(Accepted for publication July 14, 1942)

Anthracnose of cucurbits caused by *Colletotrichum lagenarium* (Pass.) Ell. and Hals. was responsible for a considerable loss in honeydew melon fruits (*Cucumis melo* L. var. *inodorus* Naud.) in the Crowley region of the Arkansas River Valley of Colorado during the growing seasons of 1939 and 1940. A serious problem from the standpoint of the shipper has been the increase of infection following packing. Large numbers of clean and apparently healthy melons became diseased, probably in transit, and, on arrival in eastern markets, frequently were either sold as culls because of anthracnose or rejected because of secondary rotting of the lesions by species of Fusarium.

In a preliminary study designed to determine whether apparently healthy melons were field-infected, 84 such melons were selected at random in fields in which anthracnose was present. These melons failed to develop any appreciable amount of anthracnose, even during incubation at high humidities and summer temperatures. Therefore, it was suspected that certain procedures used during the packing process might have been responsible for the spread of anthracnose. Shippers moved melons from the field by the truckload. A high degree of bruising and mechanical injury invariably resulted. Each truckload was then dumped into a common 2,000-gal. wash tank. The water in such a tank frequently was not replaced for days, and even weeks, at a time. From here the melons moved on rollers to brushes and under an overhead sprinkling system. They were then carried on a conveyor belt to the sorters, who first discarded the obviously diseased melons and then graded the balance.

## THE ROLE OF WASH WATER AND INJURY IN FRUIT INFECTION

In order to study the possible role of wash water in initiating new infections of injured and uninjured melon fruits, a small-scale laboratory test was conducted. Twenty melons showing active anthracnose lesions were selected. Each diseased melon was gently turned for 5 minutes in 4 liters of cold tap water in a clean, glazed 4-gallon jar. Microscopic examinations and cultural tests of the water were made after washing 4, 8, 16, and 20 infected melons. In addition 3 clean melons, each with 3 small scalpel cuts and an artificially produced bruise were rinsed in the water at the time of each examination. Three melons with similar artificial injuries were rinsed in clean tap water as a control. All melons then were held under wet canvas

[1] Paper No. 151, Scientific Journal Series, Colorado Agricultural Experiment Station.

[2] Cooperative investigation between the sections of Botany and Plant Pathology and Pathology and Bacteriology.

at summer room temperatures for 14 days. The results of this study are shown in table 1.

TABLE 1.—*The role of infested wash water and injury in infection of honeydew melon fruits by C. lagenarium*

| Infestation of wash water | | | | Infection of healthy melons | | | | |
|---|---|---|---|---|---|---|---|---|
| No. of infected melons washed | Total no. of anthracnose lesions on infected melons washed | Average no. of conidia per drop in wash water | Growth in culture plates | No. healthy melons rinsed | Total no. melons showing infection | Total no. of stabs and bruises | Total no. infections in injured areas | Total no. infections in uninjured areas |
| Control | 0 | 0 | − | 3 | 0 | 12 | 0 | 0 |
| 4 | 7 | 0.5 | + | 3 | 3 | 12 | 9 | 0 |
| 8 | 22 | 2.0 | + | 3 | 2 | 12 | 5 | 0 |
| 16 | 53 | 3.0 | + | 3 | 2 | 12 | 4 | 1[a] |
| 20 | 76 | 4.0 | + | 3 | 3 | 12 | 6 | 0 |

[a] Stem end infection. This appeared to be the result of field injury.

Table 1 indicates that as few as 4 infected melons were sufficient to infest the wash water with conidia of *Colletotrichum lagenarium*. In addition anthracnose developed only at the spots where the melons were injured. Further, where conidia were absent, as shown by the control series, no anthracnose infection developed even though the melons were injured.

## THE TOXICITY OF CHLORINE TO THE CONIDIA OF COLLETOTRICHUM LAGENARIUM

Because of the known general disinfectant action of chlorine in water, its known fungicidal[3,4,5] and non-residual properties, and its relatively low cost, solutions containing varying concentrations of chlorine were prepared from calcium hypochlorite. Since 5 minutes was noted to be the minimum interval for washing melons at the packing and shipping stations, the first problem was to determine the lowest concentration of chlorine sufficient to inactivate the conidia of *C. lagenarium* within this time period. As a preliminary procedure spores of *C. lagenarium* were taken from pure cultures of the fungus and suspended in large quantity in a few milliliters of sterile water. A loopful of this spore suspension was then transferred to each of 6 test tubes containing, respectively, 3 ml. of a solution of 10, 40, 120, 300, 600, and 1,000 parts of chlorine per million parts of water. The concentration of chlorine was determined by titration with standard sodium thiosulphate solution. After 5-minute treatments the chlorine in each solution was inactivated by the addition of an amount of either 0.1N or 0.01N

[3] Ark, P. A., and J. T. Barrett. Phytophthora rot of asparagus in California. Phytopath. 28: 754–756. 1938.

[4] Baker, Kenneth F., and F. D. Heald. Some problems concerning blue mold in relation to cleaning and packing of apples. Phytopath. 22: 879–898. 1932.

[5] Tisdale, W. B., and S. O. Hawkins. Experiments for the control of phoma rot of tomatoes. Fla. Agr. Exp. Stat. Bull. 308. 1937.

sodium thiosulphate solution[6] slightly more than that necessary to react with the amount of chlorine originally present. Drops of these solutions were then streaked on plates of potato-dextrose agar. The results of this test are shown in table 2.

TABLE 2.—*The toxicity of varying concentrations of chlorine to the conidia of Colletotrichum lagenarium*

| Concentration of chlorine (p.p.m.) | No. of conidia treated (per milliliter of suspension[a]) | Growth of conidia in culture plates | Remarks |
|---|---|---|---|
| 10 | 33,000 | + | Light growth all plates |
| 40 | 98,000 | + | Five colonies on one plate only |
| 120 | 188,000 | − | No growth |
| 300 | 138,000 | − | No growth |
| 600 | 82,000 | − | No growth |
| 1,000 | 58,000 | − | No growth |
| Control + thiosulphate | 83,000 | + | Luxuriant growth all plates |
| Control | 125,000 | + | Luxuriant growth all plates |

[a] Calculated from counts made with a ruled counting chamber.

Table 2 shows that concentrations greater than 40 parts of chlorine per million parts of water were sufficient to kill the conidia of *Colletotrichum lagenarium* suspended in water. The excellent growth of the control (untreated) spores and the spores treated with sodium thiosulphate indicated that the failure of conidia to develop in any culture plate was directly due to the chlorine treatment.

In a second study to determine the toxicity of varying concentrations of chlorine to conidia embedded in lesions on fruits, small pieces of melon tissue showing active anthracnose lesions on their surfaces were subjected to 5-minute treatments in solutions of varying concentrations of chlorine. After treatment each mass of tissue was carefully rinsed in sterile water. Following this a portion of the spore mass within a lesion was removed to a sterile water blank in order to obtain a spore suspension. Drops of this suspension were then streaked on plates of nutrient agar to determine whether the spores had been killed by the treatment. Solutions having concentrations of 40, 120, 300, 600, and 1,000 parts of chlorine per million parts of water were used in this study. It was found that a sufficient number of spores survived the treatments to give growth on the culture plates in all cases.

### THE EFFECT OF CHLORINE TREATMENTS ON HONEYDEW MELONS

The effects of 5-minute treatments in chlorine solutions of varying concentrations were noted on several crates of firm, clean, ripe honeydew melons. Commercial preparations of both sodium and calcium hypochlorite were used in obtaining 2 series of chlorine solutions of 200, 1,000, 5,000, and 10,000 parts of chlorine per million parts of water. After treatment the

[6] American Public Health Association. Standard methods of water analysis. Eighth edition. N. Y. 1936.

melons were washed in running water, held at a temperature approximating that of an iced-refrigerator car (40° F.) for 7 days, and then at room temperature for another 14 days. At the end of each time period examinations showed that none of the treatments injured the melons.

## SUMMARY

Preliminary studies regarding the increase of anthracnose of honeydew melons after harvest indicated that methods used by shippers were at fault. Washing both diseased and healthy melons in a common wash tank, coupled with rough handling of melons, appeared to be responsible for most of the increase in infection after packing.

Studies showed that as few as 4 diseased melons, when gently rinsed in 4 liters of water, were sufficient to provide a source of inoculum. When apparently healthy melons with artificially induced injuries and bruises were rinsed through this infested water, anthracnose developed only at the spots where the melons were injured.

Five-minute treatments in a solution containing 120 parts of chlorine per million parts of water proved adequate for the inactivation of the spores of *Colletotrichum lagenarium*. However, concentrations as high as 1,000 parts of chlorine per million parts of water failed to inactivate all of the conidia of *C. lagenarium* in active anthracnose lesions. Five-minute treatments in chlorine solutions with as high a concentration as 10,000 parts of chlorine per million parts of water did not injure honeydew melons.

COLORADO STATE COLLEGE OF AGRICULTURE AND MECHANIC ARTS,
FORT COLLINS, COLORADO.

# ANTHRACNOSE OF LUPINES[1]

J. L. WEIMER

(Accepted for publication June 6, 1942)

## INTRODUCTION

In March, 1939, small plants of *Lupinus angustifolius* L. were found at Quincy, Florida, that were dwarfed and malformed, with some dying or dead. Enough plants were affected in certain areas to reduce the stand considerably. This field had been planted late as part of an experiment and the plants were only 4 to 6 inches tall at the time. No such condition was noted in earlier plantings and has not been seen since, although no special search has been made. The fact that no specimens of plants thus affected or no complaints have been received leads the writer to believe that the trouble is not serious. Nevertheless, the disease is potentially capable of causing much damage to seedlings and, since no previous record of the disease on this host is known, it seems worthwhile to record here the results of such observations and experiments as have been made.

## SYMPTOMS

It was apparent that the plants in the field usually had been infected in the region of the cotyledons or through them. The cotyledons often were in the center of a lesion extending a considerable distance above and below them. In some cases the stem had been completely severed and the upper part or all of the plant killed. Other plants had a crook in the stem just above the lesion. Apparently, at one time the disease had partly girdled or greatly weakened the stem, so that it was unable to support the part above in its normal upright position. Some tops had turned at nearly a right angle to the main axis of the stem. Often there was still enough vitality in the stem to support growth so that the upper part resumed its original upright position, but left a crook (Fig. 1, A). The surface of the cankered area was brown and somewhat sunken. In some cases the surface of the lesion was slightly roughened by acervuli that had broken through the epidermis.

## ETIOLOGY

Isolations were made from several of the plants collected in the field and a fungus was obtained in pure culture. When grown on oat agar this fungus produces a minimum of mycelium and large ochraceous salmon[2]-color spore masses. The spores are unicellular, hyaline, straight, often taper slightly at both ends, and measure 3.5–6.3 × 8.4–18.9 μ (average 4.6 × 13.3 μ), which is well within the range given by Shear and Wood[3] for conidia of *Glomerella*

[1] Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Georgia Agricultural Experiment Station, Experiment, Georgia. Paper No. 97, Journal Series, Georgia Agricultural Experiment Station.

[2] Ridgway, Robert. Color standards and color nomenclature. 43 pp., 53 color plates. (Washington.) 1912.

[3] Shear, C. L., and Anna K. Wood. Studies of fungus parasites belonging to the Genus Glomerella. U. S. Dept. Agr. Bur. Pl. Ind. Bull. 252: 1–110. 1913.

*cingulata* (Stonem.) Spauld. and v. Schrenk. A comparison of this fungus with *G. cingulata* from apple has failed to reveal any differences, either in morphology or the rate at which they decay apple fruits, which would seem to justify the belief that the lupine fungus is a distinct species. Hence it is assigned to *G. cingulata.*

Thus far the writer has been able to find only two other reports of anthracnose on lupines. Seymour[4] lists *Glomerella lupinicola* Dearness on an unidentified species of lupine. Dearness has informed the writer by letter that his species has never been published and that it got into Seymour's list through an oversight. A new species of *Gloesporium, G. lupinus,* has been described by Bonder[5] in São Paulo, Brazil. It is, however, not possible from his description to determine whether he had the same species as that reported in this paper.

## INOCULATION EXPERIMENTS

Inoculation experiments, made several times, have shown beyond doubt that this fungus is capable of attacking lupine plants and causing symptoms similar to those seen in the field plants. In one experiment, set up on November 24, 1941, seedlings of *Lupinus angustifolius* were atomized with a suspension of spores in water and held in a moist chamber for 36 hours. Ten days after the inoculations were made the tips of some leaflets and petioles were dying and some infection had taken place through the cotyledons. Figure 1, B, was made on December 15, 1941. This shows a control plant at the left and two plants whose cotyledons were entirely destroyed and the stems severed. The leaf at the left has one typical lesion on the second leaflet from the right. This lesion resembles those illustrated on *L. albus* (Fig. 1, C). The disease did not make much headway in the greenhouse other than to continue to spread to a limited extent in the tissue already infected. When some of the plants were returned to the humidity chamber after a few days in the greenhouse, the disease made rapid progress. It appears from this and other experiments that, should dry weather follow an infection period, the progress of the fungus would soon be slowed down, and, if the plants had not been too severely damaged, they would resume growth and many would partially or wholly recover. During a prolonged wet spell, rainy or foggy weather sufficient to keep the plants wet, the fungus makes rapid headway and plants can be killed in a few days. Under such conditions the fungus fruits abundantly forming masses of slimy spores on the lesions, which supply an abundance of inoculum. These spores, especially while suspended in rain water, can be blown about by the wind, or carried by man or animals. Under conditions suitable for the fungus, infection takes place rapidly in the young leaflets, petioles, cotyledons, and stems.

In one experiment, spores from a culture were placed against the hypocotyl of young plants of *Lupinus angustifolius* just below the surface of the

[4] Seymour, A. B. Host index of the fungi of North America. (Cambridge, Mass.) Harvard University Press, p. 419. 1929.

[5] Bonder, G. Tremoco branco e suas molestias. Bol. Agr. São Paulo 13: 427–432. 1912.

soil to see whether infection could take place from the fungus in the soil. The plants were 10 days old and were growing in pots of sterilized soil in the greenhouse. The inoculations were made on October 29, 1941, and final notes taken on January 12, 1942. Only 2 plants out of 10 showed infection. These had large, dark-brown lesions on the hypocotyls, and many acervuli of the

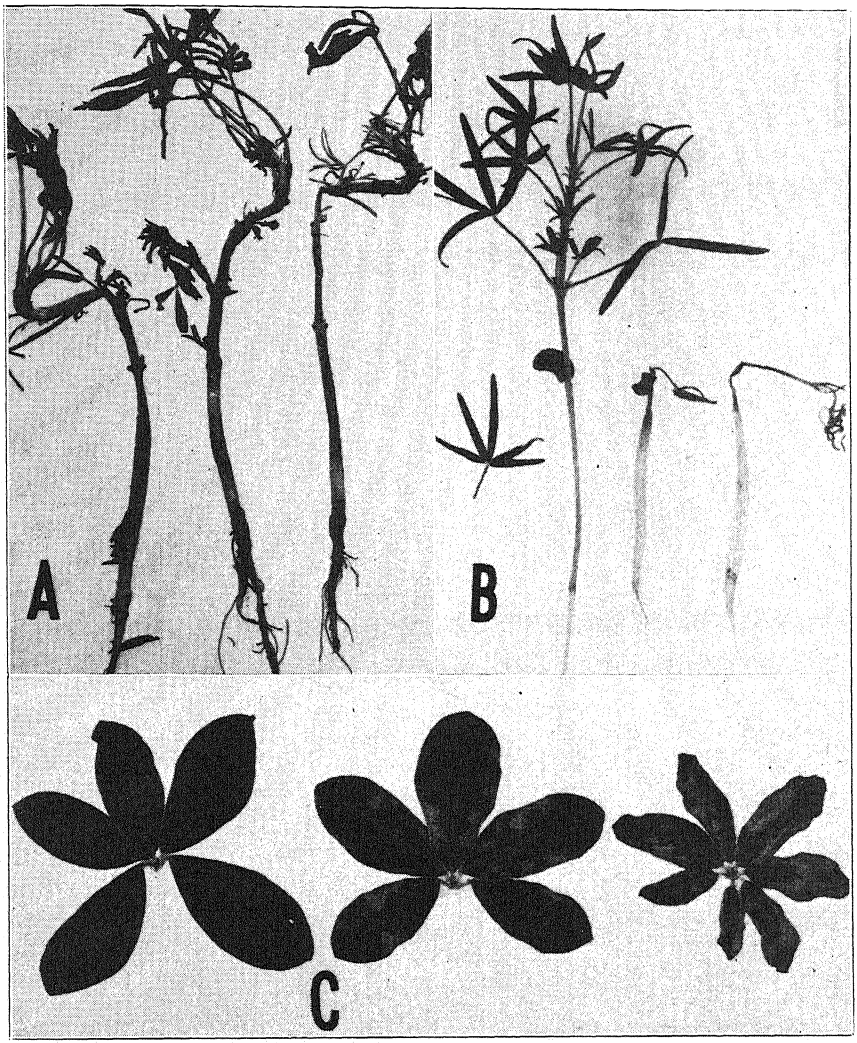



FIG. 1. Anthracnose of lupines caused by *Glomerella cingulata*. A. Three naturally infected plants of *Lupinus angustifolius* showing crooked stems and cankers at and above the cotyledons. The canker is especially conspicuous in the plant at the right. × ⅔. B. Two inoculated plants at the right in which the fungus had invaded and decayed the cotyledons and passed into the stems, severing them and causing the death of the parts of the plants above. At left a large control plant. The second leaflet from the right on the leaf at the left of the control has a lesion resembling those on *L. albus* leaves shown in C. × ⅔. C. Lesions on leaves of *L. albus* resulting from inoculation are shown on the two leaves at right. The lesions often coalesce, involving large areas of the leaflets. Control leaf at left. × ⅔.

fungus were present on their surfaces. Plants inoculated on the stems above ground failed to become infected. It seems that the stems are rather highly resistant to infection, except possibly below ground where seedlings may be infected if the soil moisture is rather high. Although this subject has not been thoroughly investigated, the experiments conducted suggest that, once the old cotyledons have fallen off and the leaflets matured fairly well, no serious damage need be expected, unless it be during an extremely long wet period.

That the leaves of *Lupinus albus* L. also can become infected was shown in an experiment in which a pot of seedlings was atomized with a heavy suspension of spores from a 4-day-old culture. The pot of plants was held in a screen house during a rainy period, and was covered by a cardboard carton. The inoculations were made on September 26, 1939, and 2 days later there were small, slightly sunken, water-soaked spots visible on most of the leaves. On the following day the tissue in these spots apparently was dead and turning gray. On the under sides of the leaves the lesions were considerably darker in color than above, but the exact details were obscured by the abundant pubescence. The spots were often up to 1 cm. in diameter, irregularly rounded, and scattered over the leaflet, but were most numerous along the margins (Fig. 1, C), where they were often confluent and had killed considerable areas. The upper sides of the lesions showed small brownish centers of undetermined origin, but assumed to be areas where the fungus hyphae were concentrated for the formation of acervuli. As the spots aged, the color darkened slightly, approaching straw color and having a narrow border of mummy brown (Ridgway) adjacent to the healthy tissue. The most severely injured plants were correspondingly retarded in growth. Unfolding terminal leaves were entirely killed. For the most part new buds were formed and growth eventually was resumed. Plants of *L. luteus* L. inoculated at the same time failed to show any infection.

These experiments indicate that anthracnose can be destructive both on *Lupinus angustifolius* and *L. albus* under high humidity and fairly high temperature conditions, especially in the seedling stage when leaves, petioles, cotyledons, and even the hypocotyls may be attacked directly and the stems be invaded by passage of the fungus into them from the adjacent more susceptible parts.

## SUMMARY

An anthracnose of lupines not previously reported in this country is described. A comparison with *Glomerella cingulata* from apple showed that the fungus from lupine was morphologically the same and hence it was considered to be that species. The fungus attacks *Lupinus angustifolius* in the seedling stage under high humidity conditions. Young leaflets, petioles, stems, and cotyledons are susceptible. The underground portion of the hypocotyl may be attacked, but the main stem is seldom invaded, except at the apex or through the cotyledons or branches. Leaves of *L. albus* were infected and severely injured. In the single test made, *L. luteus* was not infected.

# REACTION OF STRAINS AND VARIETIES OF BARLEY TO MANY PHYSIOLOGIC RACES OF STEM RUST[1,2]

F. R. IMMER, J. J. CHRISTENSEN, AND W. Q. LOEGERING

(Accepted for publication September 22, 1942)

Reid[3] found a high correlation between the reaction of barley to a composite group of races of *Puccinia graminis tritici* Eriks. and Henn. in the adult plant stage and to race 56 in the seedling stage. Brookins[4] demonstrated that in crosses involving Peatland, reaction to races 19, 36, and 56 in the seedling stage was determined by the same factor pair that conditioned reaction to a large collection of races in the adult stage.

Since attempts are being made to develop a desirable agronomic variety of barley resistant to stem rust, it would be of considerable help in the breeding program if the reaction to stem rust, especially to many physiologic races, in seedling stages could be used as a criterion of resistance in the adult stage. Consequently, such a study was made with 20 physiologic races of stem rust.

## MATERIALS AND METHODS

In this study were used two rust-resistant varieties, Peatland, C.I. 452, and Chevron, C.I. 1111; two susceptible varieties, Barbless, C.I. 5105, and Minnesota 462; and 20 hybrid strains. Nine of these strains came from a cross of Minnesota 462 × Peatland, and 11 from Barbless × Peatland. All these lines had proved to be resistant when grown in rod rows and tested against a composite of many races of stem rust in the field.

The 24 varieties and strains of barley were tested for seedling reaction to 19 races of *Puccinia graminis tritici* and one collection of *P. graminis secalis* Eriks. and Henn. in the greenhouse in 1940 and 1941. The following races of *P. graminis tritici* were used: 10, 14, 15, 17, 19, 21, 24, 29, 34, 40, 48, 49, 53, 55, 56, 59, 97, 139, and 147; these were furnished by the Division of Plant Disease Control of the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station.

Seed of each variety or strain was planted in 4-in. flowerpots and inoculated with a single race of rust. The reaction of each variety or strain to

[1] Published as Scientific Journal Series Paper No. 2014 of the Minnesota Agricultural Experiment Station.

[2] Cooperative investigations between the Minnesota Agricultural Experiment Station and the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

[3] Reid, D. A. A study of the inheritance of seedling and mature plant reaction to *Puccinia graminis tritici* in a cross of Wisconsin 38 × Peatland barley. M.S. thesis. University of Minnesota. 1938.

[4] Brookins, W. W. Linkage relations of the factors determining reactions to stem rust in barley. Ph.D. thesis. University of Minnesota. 1940.

each of the races of rust was recorded in the manner described by Stakman and Levine.[5]

## EXPERIMENTAL RESULTS

The two varieties, Barbless and Minn. 462, susceptible to collections of many races of stem rust in the mature plant stage in the field, also were susceptible to all races in the seedling stage. The 22 varieties and strains of barley, resistant in the field, were resistant to 18 of the 19 races in the seedling stage. Seedlings of all varieties and strains of barley were susceptible to physiologic race 29. This particular collection of race 29 was obtained from aecia on barberry.

In order to determine the reaction of adult plants to race 29, rusted seedlings from a number of hybrid lines were allowed to grow and then were reinoculated at heading time in the spring of 1941. Although the rust reactions were obtained for only 6 hybrid strains in the adult stage, all the plants of these hybrids were again susceptible.

All varieties and strains of barley were resistant to the one collection of *Puccinia graminis secalis* to which they were tested.

These results indicate that the varieties of barley susceptible in the seedling stage to certain races of rust also are susceptible in the adult stage. The reaction in the adult stage and seedling stage was the same for all races tested, indicating that the reaction is of a physiologic nature.

## SUMMARY

Two varieties of barley, normally susceptible to stem rust under field conditions, and two varieties and 20 hybrids, normally resistant in the field, were tested in the seedling stage for reaction to 19 physiologic races of *Puccinia graminis tritici* and one collection of *P. graminis secalis*.

The varieties and hybrids that were resistant in the field proved resistant to all physiologic races of *Puccinia graminis tritici*, except race 29, in the seedling stage, and were resistant also to the collection of *P. graminis secalis*. The two varieties susceptible in the field were susceptible to all races of *P. graminis tritici* in the seedling stage but resistant to the collection of *P. graminis secalis*.

All varieties and strains of barley tested were susceptible to race 29 in the seedling stage. Six of these strains were tested in the adult stage and found to be susceptible.

Seedling reaction to rust can be used to eliminate lines of barley which are susceptible in the adult stage.

UNIVERSITY FARM, ST. PAUL, MINN.

[5] Stakman, E. C., and M. N. Levine. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Minn. Agr. Exp. Stat. Bull. 8. 1922.

## PHYTOPATHOLOGICAL NOTES

*A Disease of Apple Grafts and Layers Caused by a Rhizoctonia.*—A disease of underground portions of stems of apple nursery trees has been causing considerable loss in the experimental nursery at the Maryland Agricultural Experiment Station at College Park, Maryland. The methods used there for propagating apple trees on their own roots are: 1, Planting grafts in a furrow and drawing the soil to the young shoots as they grow, thus producing etiolated shoots that readily strike root; 2, pinning down in a furrow and covering with soil the tops of 2-year-old nursery trees and drawing the soil to the young shoots as they emerge from the buried tops, thus providing etiolated stems that readily form roots. Either the graft or the layer method of own-root propagation has the disadvantage, from the disease standpoint, of exposing young shoots that have the characteristics of herbaceous material to the attack of soil fungi of the damping-off type. In 1936, some losses occurred in the experimental apple nursery. In 1937 nearly all of a block of 10,000 grafted plants were killed, and in the layered plants there was a loss of about 10 per cent. In 1938, nearly 12,000 grafted plants were killed.

A similar disease has been causing some loss on apple seedlings (Fig. 1, C) at the U. S. Bureau of Plant Industry Station, Beltsville, Maryland. In certain places in the nursery, where conditions were favorable and the pathogen was present, a large percentage of the young seedlings were killed when 2 to 4 inches high.

The disease first becomes manifest as definite and distinct lesions on the young underground stem of a graft or layer. These lesions may advance very rapidly and soon girdle the stem, giving a damping-off effect (Fig. 1). In less severe cases the lesions may advance more slowly, and the shoot may grow to be a foot or more high before it is girdled and dies. Many times, especially when conditions are not favorable for infection, a large number of lesions occur on the underground stems; but they heal, and the next year the scars from healed lesions are very characteristic (Fig. 1, A, B). Another symptom usually present is the adherence of fungus filaments with enmeshed particles of soil to the leaves and stems (Fig. 1, A), in contact with the soil, thus producing a cobweb-like effect. This symptom is similar to that of a related disease on holly cuttings.[1]

Some apple varieties are much more susceptible than others; but more work must be done before attempting to list varieties in the order of their susceptibility or resistance. It was observed, however, that U.S.D.A. clone No. 227[2] showed fewer and smaller lesions the second year after inoculation of layer shoots than did any other of a number of clones or varieties.

When losses showed up in June, 1938, the writers began searching for the cause of the trouble. No reference to such a disturbance on grafts or layers

[1] Cooley, J. S. Defoliation of American holly cuttings by Rhizoctonia. Phytopath. 32: 905–909. 1942.

[2] This clone was introduced by Guy E. Yerkes for use as an understock.

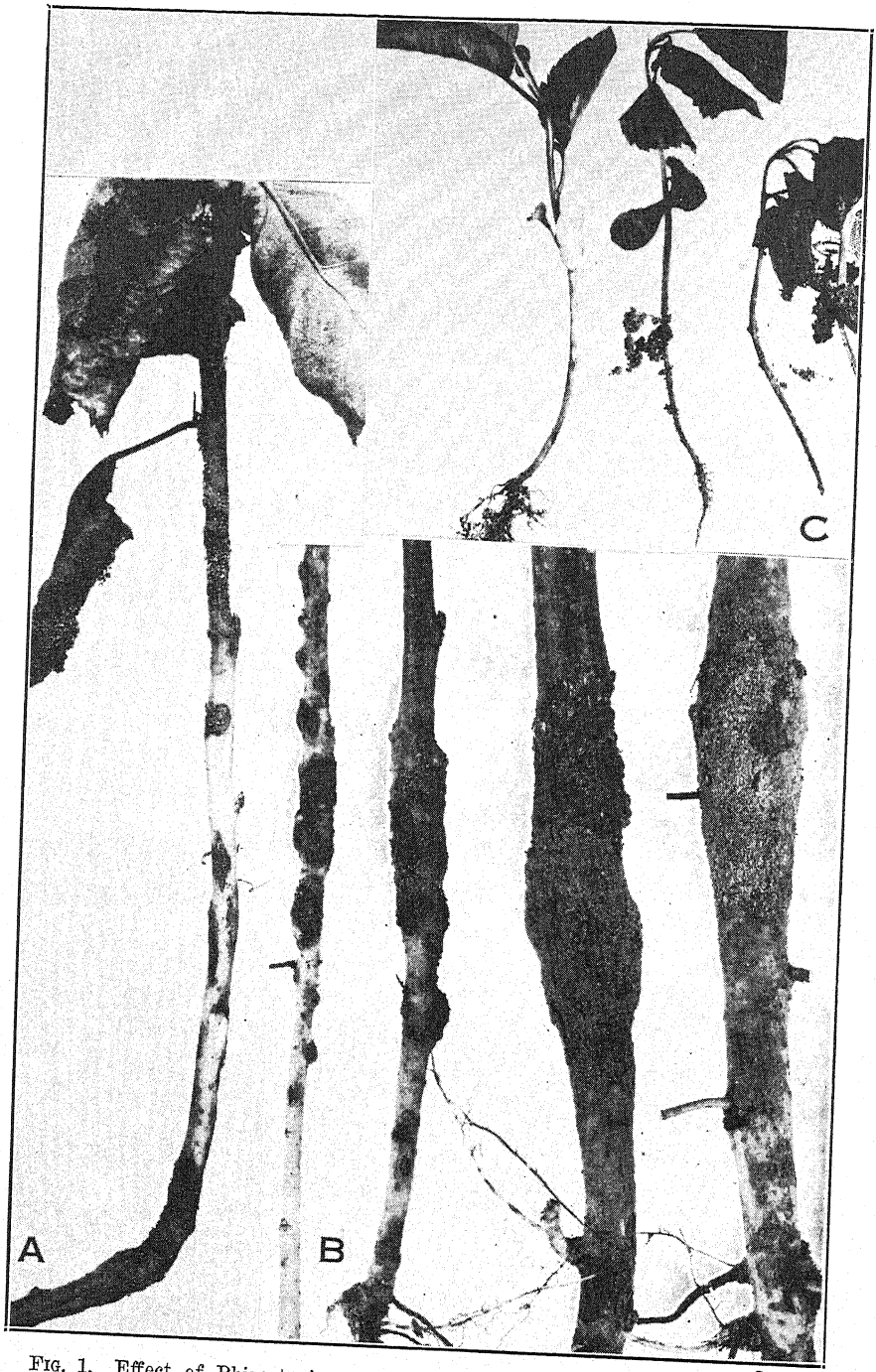



FIG. 1. Effect of Rhizoctonia on shoots of apple layers and on apple seedlings. A. Lesions, some causing girdling, on shoots of apple layers, also enmeshed grains of sand on the under side of affected leaves. B. Healing lesions and hypertrophy on layer shoots of apple trees the second summer after infection. C. Different stages in the killing of apple seedlings.

has been found in the literature. E. A. Siegler,[3] over a period of years since 1920, made observations and isolations from lesions on roots of apple and peach seedlings. He consistently isolated a species of Rhizoctonia from affected plants and has repeatedly observed sclerotia of Rhizoctonia on apple seedlings from commercial apple seedling nurseries. J. Perlberger[4] reported apple and other nursery trees of deciduous fruits affected with *Rhizoctonia bataticola* (Taub.) Butler group B. The disease, he says, sometimes destroys up to 60 per cent of the young trees in Palestine.

From the margin of dead and living tissue of active lesions of affected apple plants the writers have consistently isolated a species of *Rhizoctonia*.[5]

Flasks of sterilized oats inoculated with this fungus were used in the field as inoculum on young layer shoots. When this inoculum was placed underground around young layer shoots severe infection took place. Lesions were produced that were similar to those from which the inoculum was taken and a fungus apparently identical with the original inoculum was reisolated. In some cases where the conditions were favorable for infection, all the shoots in plots of 20 or more were completely killed, while the noninoculated checks in the rest of the nursery row showed no injury or only a trace.

An experiment looking to the control of this disease on grafts and seedlings in the nursery was carried out during the summer of 1940. Copper oxide dust in several concentrations diluted with talc, and also Bordeaux mixture and other fungicides were applied in the nursery on inoculated soil just prior to drawing the soil to the trees. The results of this work were inconclusive because conditions did not favor severe infection after the experiment was begun. The percentage of infections on the inoculated and untreated plots was so low that no conclusion could be drawn as to the effectiveness of any of the treatments.—J. S. COOLEY AND F. B. LINCOLN, Bureau of Plant Industry Station, Beltsville, Maryland and University of Maryland, College Park, Maryland.

*A New Parasite of Tomatoes.*—During the early fall of 1942, County Agent Kirk at Casper, Wyoming, sent in a few specimens of tomatoes with an infection, later identified as broom-rape, (*Orobanche ludoviciana* Nutt.).

The grower of these tomatoes, J. K. Kear, living near Casper, reported that this parasite was present on 50 plants out of 700 plants in one lot of the variety, 10-ton Rutger. Some of the infected plants were stunted while others were not and the tomato yield was light.

This was the first crop to be grown on the land since it had been reclaimed from native sod. No doubt the parasite had been active on sagebrush and possibly on other perennials where it is commonly found. How-

[3] Unpublished notes.

[4] Perlberger, J. *Rhizoctonia bataticola* (Taub.) Butler, in deciduous fruit-tree nurseries in Palestine. Palest, J. Bot. Hort. Sci. 1: 37–51. 1937. (Hebrew) abs. R. A. M. Vol. 16, Part 10, October, 1937, pp. 683, 684 Kew.

[5] A culture of this fungus was sent to Ernest Wright, who is making a study of forest-tree seedling diseases. He gave the opinion that the fungus is *Rhizoctonia solani*.

ever, it is of significance that *Orobanche* was found on an annual plant rather than on a perennial one, and it is thought to be novel in that respect.

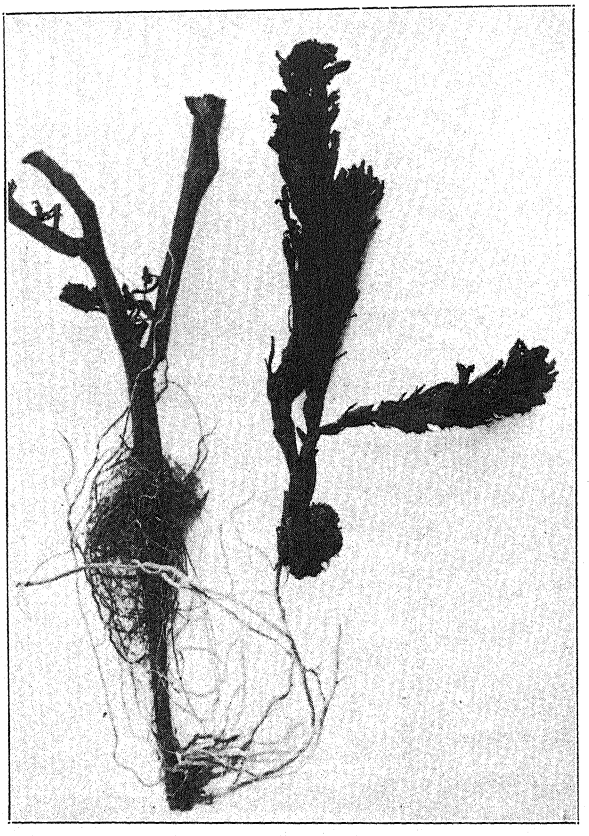


FIG. 1. Tomato plant with its broom-rape parasite.

The accompanying cut shows the relative size of the parasite and its point of attachment.—G. H. STARR, Wyoming Agricultural Experiment Station, Laramie, Wyoming.

*Two Items of Pathological History from California.*—Evidence has been found in California agricultural literature to indicate considerably earlier dates for certain events of importance in plant pathology, than those ascribed to them in a recently published text book.[1] One of these relates to the use of copper as a fungicide.

"The use of copper salts as fungicides was first made popular in 1873, when copper sulphate solution was used to disinfect wheat seed carrying the bunt organism."

(Melhus and Kent, p. 99). Early volumes of *Trans. Cal. State Agr. Soc.* contain many references to treatment of seed wheat with bluestone to prevent

[1] Melhus, Irving E., and George C. Kent. Elements of Plant Pathology. Macmillan Company (N. Y.). 493 pages. 1939.

smut, as, for instance, the article in volume 1 (1858), which includes (p. 343) the following statement from M. Walthall, Jr. of Stockton.

"Seed wheat I wash in clear water, if there be smut in it, and soak it in very strong blue stone water—say one pound to eight gallons—about twenty minutes, and then roll it in fresh slaked lime, and sack it for use."

The short-lived California *Culturist* (1858–1861) also contains many references to the copper sulphate treatment, including (Vol. 1, p. 350, 1858) an account of an experiment in which the efficacy of the method was convincingly demonstrated. In an address at the State Fair of 1860, John Bidwell of Chico, one of California's most reliable early observers, stated (*Transactions,* 1860, p. 325) that he had been familiar with grain growing in the Sacramento Valley since 1841, and that smut (bunt) first appeared there suddenly in 1854 in a field sown with Australian seed. He endorsed the bluestone treatment, which, by 1860, was apparently almost universal with California wheat farmers. In 1867 a San Francisco firm made the statement (*Transactions,* 1867, p. 217)

"During the years 1853, 1854, 1855 and 1856 our grain was badly injured by smut, which the use of blue vitriol has remedied."

Regarding peach leaf curl, Melhus and Kent state, page 298,

"The pathogen, (*Taphrina deformans*) probably was introduced into the United States in the early Eighties of the last century."

No reference to the source of this information is given, but one wonders if it may have originated in the statement by Owens,[2] p. 262,

"The writer has been unable to find any mention of this disease in the American literature prior to 1883."

Many early American books on fruit growing, like Downing's "Fruits and Fruit Trees of America" (1845), describe peach leaf curl. In California Harkness[3] states, "I first observed this in the State in 1855." From 1858 on, the disease was a very common subject of discussion in the horticultural press of California, the natural inference being that the pathogen had been introduced into the State on some of the first peach nursery trees, which were brought in about 1850, and frequently thereafter. The *Transactions* for 1860 (p. 283) contained an essay on the subject of curl leaf which concludes,

"It is caused by the sudden change of the atmosphere from heat to cold while the foliage is in young and tender state of growth. As curl is caused by the action of the atmosphere, and that being governed by the Creator, I believe it to be a matter of impossibility for man to prevent the ravages of curl, to any great extent."

Harkness,[4] in a later article, made the dubious suggestion that *P. deformans* is identical with an indigenous fungus now called *T. Aesculi* (E. and E.)

[2] Owens, Chas. E. Principles of Plant Pathology. John Wiley and Sons, Inc. (N. Y.). 629 pages. 1928.

[3] Harkness, H. W. Fungi injurious to fruit trees. Rpt. California State Bd. Hort. Commsrs. **1883:** 51–59. 1883.

[4] Harkness, H. W. The curled leaf. Zoe. **1:** 87–88. 1890.

Giesenhagen, on California buckeye. A different light on the history of peach leaf curl in California is thrown by a statement by John T. Strentzel,[5] one of the State's most respected early horticulturists, who arrived in 1850 and took a special and very intelligent interest in fungus diseases.

"In California, previous to the year 1852, a few peach trees were found about the Missions, around Los Angeles, the fruit of small size, white or yellow, globular, with a deep suture, *the trees badly affected with curl leaf.*" (The italic is ours). "Later, when budded trees, imported trees, began to be plenty, their vigorous growth and luxuriant foliage were characteristics distinguishing them from the so-called Mission peach. A few years later the curl began to appear also on budded trees."

It would be interesting to know the history of this disease in Mexico, occupied by Spanish settlers for 250 years before colonizing Alta California. It is commonly assumed that their fruit trees (except the fig, olive and vine) were all seedlings, but it may not have been impossible for them to have transported living trees on occasion. C. W. Reed of Yolo County (*Transactions,* 1867, p. 231) in 1856, "imported about eighty thousand trees, mostly grafted," and that was more than ten years before the opening of the transcontinental railroad.—RALPH E. SMITH, Division of Plant Pathology, University of California, Berkeley.

[5] Strentzel, John T. The peach tree and its diseases. Pacific Rural Press **20**: 182. 1880.

# TWO SPECIES OF PYTHIUM OCCURRING IN SOUTHERN STATES

CHARLES DRECHSLER

(Accepted for publication July 18, 1942)

In several recent papers (19, 20, 21, 22) supplementary discussion and illustrations have been supplied for 11 of the 15 species of *Pythium* that I presented as new in diagnoses published 12 years ago (14). Similar treatment is accorded herein to *P. myriotylum*, one of the remaining 4 species, which occurs in some southern States as a parasite on economic plants. In addition, another fungus, likewise of southern origin, though not intimately akin to *P. myriotylum*, is described as a new member of the same genus. For facility of comparison all figures relating to the formation of oospores in both fungi are given at the same magnification (*i.e.*, ×1000) used for such illustrations in previous accounts; and similar magnification is employed for illustrations showing early stages in the germination of oospores. Owing to limitations of space, the figures pertaining to asexual reproduction by sporangia of mycelial origin are again given at half this magnification (*i.e.*, ×500), as are also the equally rangy figures showing zoosporangia produced in the later stages of oospore germination. The smaller scale of magnification applies no less advantageously to the drawings of the rangy branching clusters of appressoria in *P. myriotylum*. The elaborate intrication of hyphae, often observable when either of the 2 fungi is encountered by any one of several other oomycetes associated with root rot, also pertains to the vegetative stage; but as it entails frequently a complicated arrangement of parts, the larger scale of magnification has appeared generally preferable for the drawings relating to antagonistic or parasitic action in dual cultures.

## PYTHIUM MYRIOTYLUM

In May, 1924, the late Dr. W. A. Orton, then in charge of investigations on truck crop diseases in the Bureau of Plant Industry, submitted to me a tomato (*Lycopersicon esculentum* Mill.) plant from South Carolina, with instructions to isolate from its discolored rootlets whatever parasitic fungi might be present in them. Among the several oomycetes obtained in pure culture from the affected material was a species of *Pythium*, which, except for its less massive development of sporangial lobules and its much readier production of zoospores in irrigated agar preparations, strongly resembled an allied form that I had often isolated from discolored roots of maize (*Zea mays* L.). The same species of *Pythium* was recognized the next month in a culture derived from a decaying cucumber (*Cucumis sativus* L.) fruit selected in Philadelphia as being illustrative of damage observed in a carload lot of cucumbers shipped from South Carolina. When the fungus was introduced by wound inoculation into sound marketable cucumbers, a rapid watery decay resulted, much like the "cottony leak" attributable to *P.*

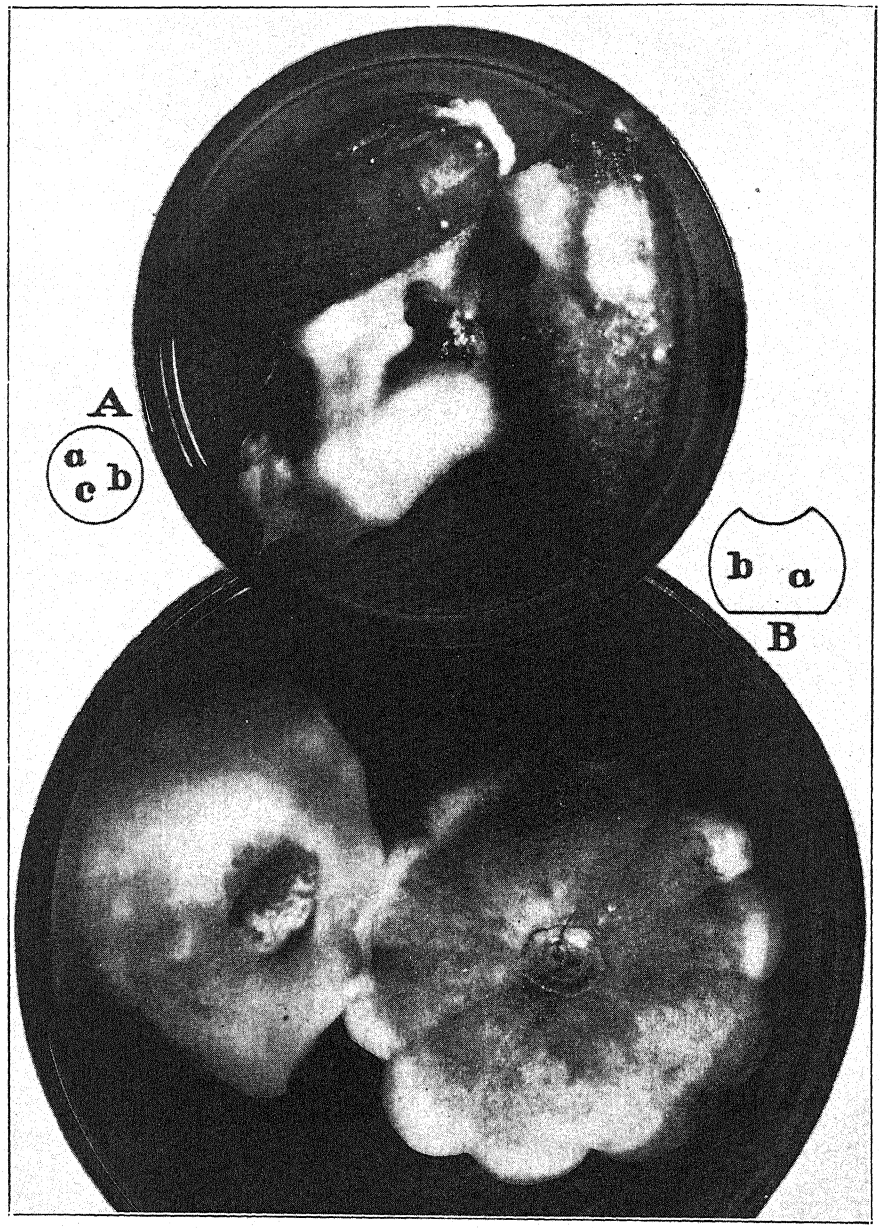



FIG. 1. A. Three cucumbers (a, b, c) infected with *Pythium myriotylum;* photographed 5 days after inoculation of specimen c was accomplished by placing on its uninjured epidermis a sizable slab taken from a young maize-meal-agar plate culture of the fungus, and then incubating in a damp chamber at 28° C. The parasite during the period of incubation spontaneously invaded the neighboring specimens a and b; about $\times \frac{2}{3}$. B. Two specimens of pattypan squash (a, b), each inoculated with *P. myriotylum* through an incision at the flower scar; extensive infection of the older and harder specimen (a) is shown only in a water-soaked area reaching nearly to the scalloped margin; the younger and tenderer specimen (b), invaded throughout, has become clothed in aerial mycelium; about $\times \frac{1}{2}$.

*butleri* Subr. For that matter, wounding was found unnecessary for successful infection. Under humid conditions and at fairly high temperatures (25 to 35° C.) mere application of sizable slabs of agar medium, newly permeated with mycelium, to uninjured epidermis consistently led to watery decay of the vegetable, and to its envelopment in abundant cottony mycelium (Fig. 1, A, a, b, c), which, on encountering adjacent cucumbers, caused their destruction in turn. Although this strong tendency toward aerial parasitism on cucumbers had been found alien to the maize-root fungus (9) its recognition as a physiological feature regularly associated with less pronounced sporangial lobulation and readier zoospore development required confirmation in additional cultures of separate origin.

Eleven such cultures, among a larger number referable to *Pythium butleri* and *P. acanthicum* Drechsl., were isolated early in June, 1925, from separate watermelon (*Citrullus vulgaris* Schrad.) fruits found partly destroyed by blossom-end rot in fields near Leesburg and Bradenton, Florida, and Thomasville, Georgia. A few weeks later 5 other cultures of the fungus under consideration were isolated from separate eggplant (*Solanum melongena* L.) fruits found damaged in fields near Bradenton by a ground rot closely resembling the "cottony leak" in the 18 similar specimens which yielded the cultures of *P. butleri* that, in a report (10) published at the time, were referred to *P. aphanidermatum* (Eds.) Fitzp. Under warm, humid conditions, introduction of the fungus into watermelon and eggplant fruits by wound inoculation always eventuated in watery decay of these vegetable products. The uninjured epidermis of both these fruits proved resistant to invasion, though in a few instances blossom-end infection, quite similar to spontaneous infection, resulted when a slab of agar medium newly permeated with mycelium was applied to the flower scar for 2 or 3 days. Eggplant fruits invaded by the fungus, whether from artificial inoculation or spontaneous infection, often afforded a copious growth of aerial mycelium (Fig. 2, A, B), but infected watermelons, with their hard rind of strongly indurated tissues, permitted no external development of the parasite. When slabs of agar medium, newly permeated with mycelium derived from any of the 16 cultures isolated from watermelon and eggplant fruits, were applied to uninjured cucumbers, watery decay promptly ensued, with abundant production of cottony aerial mycelium; and the aerial mycelium, on reaching other uninjured cucumbers, would closely invest them, penetrate into them, and thus destroy them in turn. In view of their capacity for aerial parasitism, as well as of their rather moderate sporangial lobulation and their ready production of zoospores in irrigated agar preparations, these 16 cultures, while generally resembling the maize parasite, differed from it in the same particulars as the 2 cultures previously isolated from material originating in South Carolina. The 18 cultures, therefore, were considered to belong to a separate species, which, in 1930, I described under the binomial *P. myriotylum,* 2 years after I had presented the maize parasite as *P. arrhenomanes* (12).

Thus at the time of its description *Pythium myriotylum* had become known to me as a root-rotting parasite only through a single encounter. Later, in 1931, I recognized the species in a number of cultures received from S. C. J. Jochems of the Deli Proefstation at Medan, Sumatra, where they had been isolated in connection with studies on a serious foot rot most

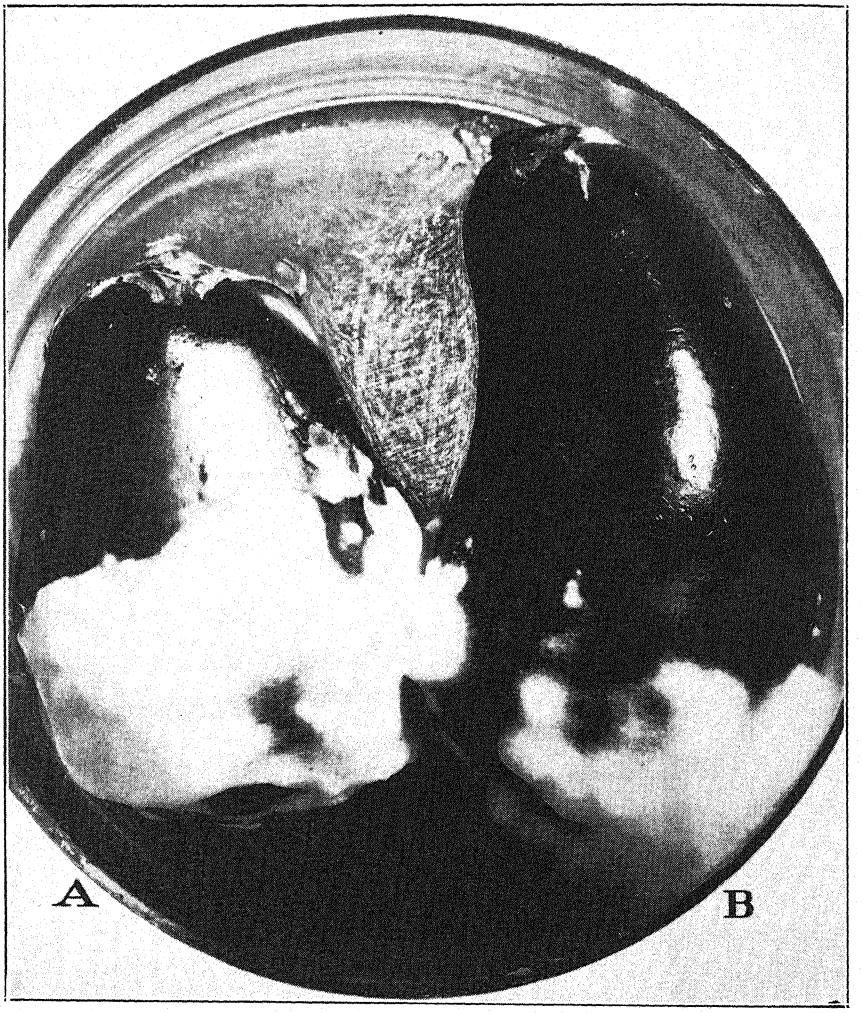



FIG. 2. Two eggplant fruits (A and B) infected with *Pythium myriotylum;* photographed 5 days after each had been inoculated by introducing the fungus into an incision at the stylar scar; incubated in a damp chamber at 28° C.; about $\times \frac{1}{2}$.

often attacking tobacco (*Nicotiana tabacum* L.) seedlings newly transplanted from seedbed to field. In an extended account, published in 1927, Jochems (25) had given a full description of the disease in question, denominating it as "parasitaire Stengelverbranding" or "parasitic stem-burn," and had indicated as causal organisms 4 unidentified species of

*Pythium,* which he designated provisionally by the letters A, B, C, D, though holding that *P. debaryanum* Hesse and *P. aphanidermatum* were probably included among them. Somewhat earlier, to be sure, in a list of plant diseases and pests attacking cultivated plants in the Dutch East Indies (23), responsibility for causation of "Stengelverbranding" was attributed to species of *Pythium* cited under the 4 binomials *P. debaryanum, P. butleri, P. polyandrum,* and *P. nicotianae.* Descriptions applicable to the latter 2 binomials have never been supplied. However, in 1934, Meurs (27) made known that the fungus cited by van Hall (23) in 1925 as *P. polyandrum* was the same as *P. myriotylum,* which species, together with 2 congeneric forms, he had discovered in scores of cultures isolated by him from diseased tissues of tobacco plants found affected with stemburn in Sumatra during the period from 1929 to 1932. With respect to the nomenclature of the parasite, Meurs dismissed van Hall's binomial from consideration as a *nomen nudum.* In the meantime, incidentally, the specific component of that binomial—or rather an orthographic variant not considered different according to established rules of botanical nomenclature (3, p. 22, Art. 70, Note 4) —had been applied by Sideris (32) to a closely related and unquestionably congeneric fungus which he described as new under the binomial *Nematosporangium polyandron,* but which Rands and Dopp (30) have subsequently found referable to *P. arrhenomanes.*

Further indication that *Pythium myriotylum* occurs in affected vegetative parts of crop plants was provided when the fungus came to light in one of 3 pure cultures submitted to me for identification in March, 1940, by Dr. M. N. Walker of the Watermelon and Ornamental Field Laboratory at Leesburg, Fla., where they had been isolated from watermelon seedlings (35). Recently, too, the parasite has been reported unambiguously by additional observers in foreign lands. In 1936 Park (29) noted its occurrence as the cause of a soft rot affecting ginger (*Zingiber officinale* Rosc.) imported from India and planted in Ceylon. Subsequently Uppal (33) stated that the fungus causing soft rot of ginger in Surat had been determined to be *P. myriotylum.* According to Wager (34) *P. myriotylum,* together with 2 congeneric forms, was isolated once from papaw (*Carica papaya* L.) plants affected with "foot rot" in South Africa. Though a species of *Pythium,* isolated in Sierra Leone from garden bean (*Phaseolus vulgaris* L.) plants affected with wilt, was determined only rather indefinitely as being near *P. myriotylum,* Deighton's account (7) of the manner in which under moist conditions its woolly mycelium soon covered the stems of infected plants and then spread over the ground to attack other seedlings in its path, very aptly describes the growth habit of *P. myriotylum.*

That *Pythium myriotylum* may really have been the species encountered by Deighton appears the more likely from its aggressive parasitism on snap beans under experimental conditions (Fig. 3, A, B). Such parasitism on the uninjured immature edible pods of garden beans was reported in 1927 by Harter and Whitney (24), who found the fungus capable of bringing

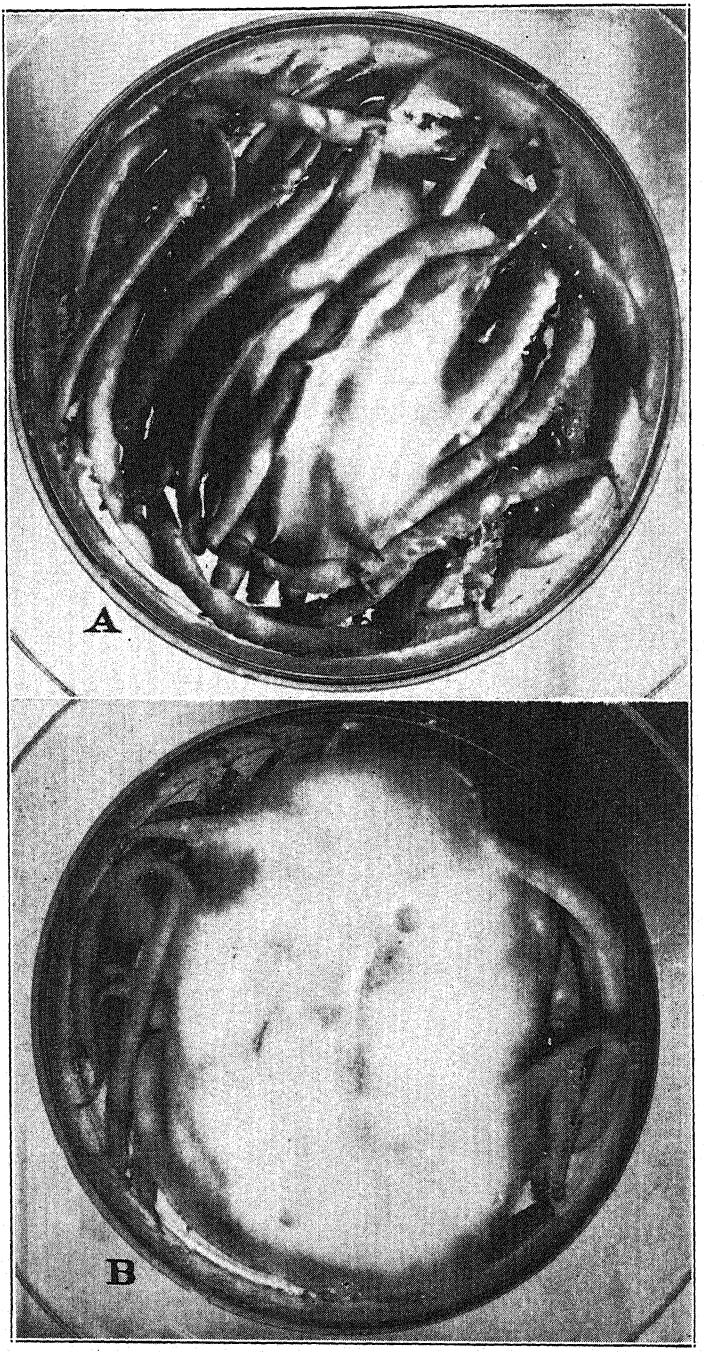



FIG. 3. String beans showing progressive attack by *Pythium myriotylum*, after a sizable slab of maize-meal-agar medium, permeated with mycelium, had been placed on the uninjured pods in the center of the dish. A. Photographed after incubation at 28° C. for 3 days. B. Same material photographed 2 days later, that is, after incubation at 28° for 5 days; about × ½.

about a decay of this vegetable product very similar to the "nesting" caused spontaneously in transit by the congeneric *P. butleri,* designated by them as *P. aphanidermatum.* Like cottony leak of cucumbers, the decay of bulked snap beans caused by *P. myriotylum* is accompanied by production of profuse cottony mycelium. Profuse aerial mycelium is usually produced also in the destruction of summer squashes (*Cucurbita pepo* L.) under moist conditions, following artificial inoculation with the fungus; the larger, older, and somewhat harder specimens of this vegetable (Fig. 1, B, a) permitting such production less promptly than younger and tenderer specimens (Fig. 1, B, b). Wound inoculation of the parasite into muskmelons (*Cucumis melo* L.) and Cassaba melons (*C. melo* var. *inodorus* Naudin) ordinarily leads to no conspicuous cottony development externally, though the flesh within the hard rind often becomes extensively permeated with mycelium, and, though aerial mycelium often is produced within the central cavity.

In pure culture, especially at rather high temperatures, *Pythium myriotylum* extends its mycelium more rapidly than most allied forms. Accordingly, it must be reckoned, together with such familiar pathogenic species as *P. ultimum* and *P. butleri,* among the fastest-growing of fungi. Further similarity to the 2 species mentioned is apparent not only in the somewhat coarse texture of its mycelium, but also in the disorderly arrangement and promiscuous ramification of its mycelial filaments. On maize-meal-agar media, or on other media not rich in nutrients, aerial mycelium may be produced in only small quantity, particularly if the overlying or surrounding air is deficient in moisture. However, if a humid atmosphere is maintained in a Petri dish containing a plate culture prepared with a medium rich in nutrients as, for example, Lima-bean agar, the dish often becomes completely filled with aerial mycelium of such density and firmness that the basal part of the container may be held up loosely suspended from the lid.

Microscopic examination of the under side of a Petri-dish culture prepared with maize-meal agar, usually shows everywhere a generous scattering of knob-like appressoria in contact with the glass floor. When thus produced at the interface of agar medium and glass, these organs commonly measure about 7 or 8 μ in diameter, and consist of swollen terminations on rather short lateral branches, though their development as lateral protuberances on longer hyphal elements is not infrequent (Fig. 4, A, B). With respect to the abundance of such intramatrical appressoria, *P. myriotylum* appears comparable to *P. butleri* and other strongly parasitic members of the genus. However, with respect to the abundance of its production of appressoria on the surface of solid objects encountered by its aerial mycelium, the fungus is unrivalled by any congeneric form known to me. In Petri-dish cultures with copious aerial development the ceiling of the container often is beset everywhere with dense clusters of appressoria borne on systems of closely ramifying branches (Fig. 4, C, D). The adhesive organs formed here usually measure from 8 to 11 μ in diameter, and thus are notice-

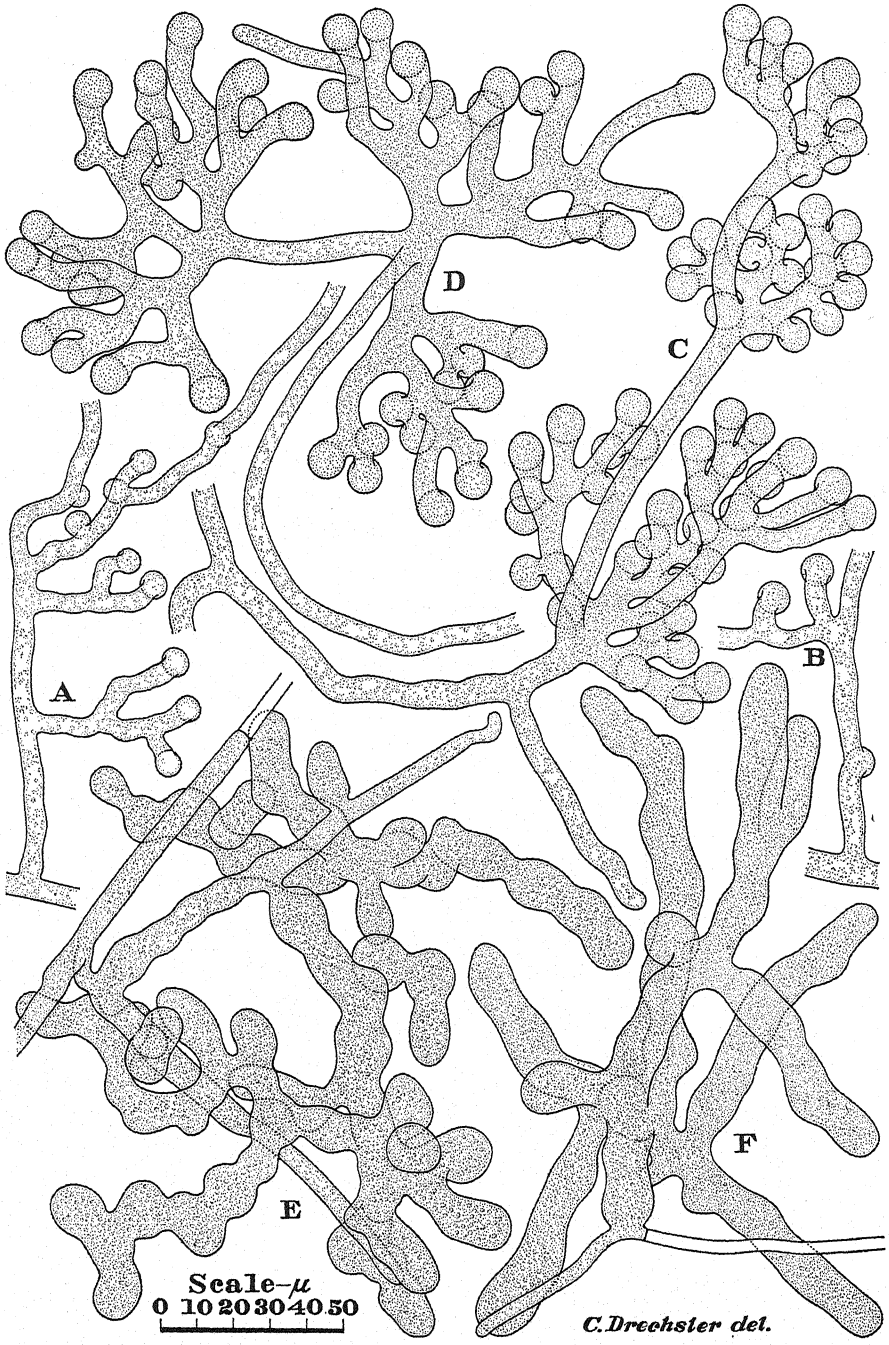



FIG. 4. Vegetative and asexual reproductive structures of *Pythium myriotylum* produced in Petri-dish cultures; drawn to a uniform magnification with the aid of a camera lucida; ×500 throughout. A, B. Portions of submerged mycelium bearing appressoria in contact with floor of container. C, D. Portions of aerial mycelium bearing appressoria in contact with ceiling of container. E, F. Systems of swollen sporangial branches formed on surface of maize-meal-agar medium.

ably larger than the homologous organs produced under the medium. Unquestionably the abundant development of aerial appressoria by the fungus is related to its capacity to operate as an aerial parasite in the causation of such trouble in bulked vegetables as cottony leak or nesting.

When grown in plate cultures on maize-meal agar or Lima-bean agar, *Pythium myriotylum* often produces, especially in areas where the surface of the medium is moist with water of condensation, more or less branched systems of swollen digitate elements (Fig. 4, E, F; Fig. 5, A). Like the homologous, though usually more extensive, ramifying complexes produced under similar conditions by *P. butleri* and *P. complens* Fischer (=*P. torulosum* Coker and Patterson), these branched systems, on being bathed in fresh water, give rise to evacuation tubes and undergo transformation into zoosporangia; the smaller systems usually being converted into single zoosporangia, the more extensive ones into plural zoosporangia. Development of swollen branches takes place in some measure also when young undifferentiated mycelium is supplied with fresh water under conditions suitable for prompt production of zoospores; noticeably swollen elements being formed when, for example, small pieces of a newly invaded watermelon or cucumber, or small slabs excised from a young Lima-bean agar culture, are transferred to a shallow layer of water. Though some of the sporangia partitioned off as separate reproductive units in such a preparation may include perhaps a dozen swollen branches (Fig. 5, B) together with hyphal parts of smaller volume and an evacuation tube (Fig. 5, B, t), many other sporangia may consist of a rather long portion of unmodified filament together with only a few swollen branches (Fig. 5, C, D). Indeed, many of the smaller sporangia may include no perceptibly swollen elements whatever (Fig. 5, E). Although an evacuation tube arising from a swollen part (Fig. 5, B, t; F, t), or one arising laterally from a rather wide undifferentiated hyphal part (Fig. 5, E, t), is usually recognizable as a special structure, similar distinctiveness is ordinarily absent when an evacuation tube is formed through prolongation of an outwardly unmodified branch or filament (Fig. 5, C, D). Functional frustration of evacuation tubes seems rather more frequent here than in most congeneric forms; the frustrated tube soon being walled off in whole or in part by deposition of a septum (Fig. 5, F, a), and a new evacuation tube thereupon being put forth from some other position (Fig. 5, F, t).

Zoospore formation in *Pythium myriotylum* is associated with the sequence of internal changes usual for members of the genus. As the evacuation tube attains definitive length, and forms a hyaline cap of dehiscence (Fig. 5, B, t), the protoplasmic contents of the sporangium show increasing vacuolization. The hyaline cap suddenly yields to become inflated into a vesicular membrane as the migrating sporangial contents accumulate within it. Once the migration is completed the undifferentiated granular mass (Fig. 5, C; F, t) undergoes division, and in the course of about 20 or 25 minutes is transformed into laterally biciliate, motile zoospores (Fig. 5, D,

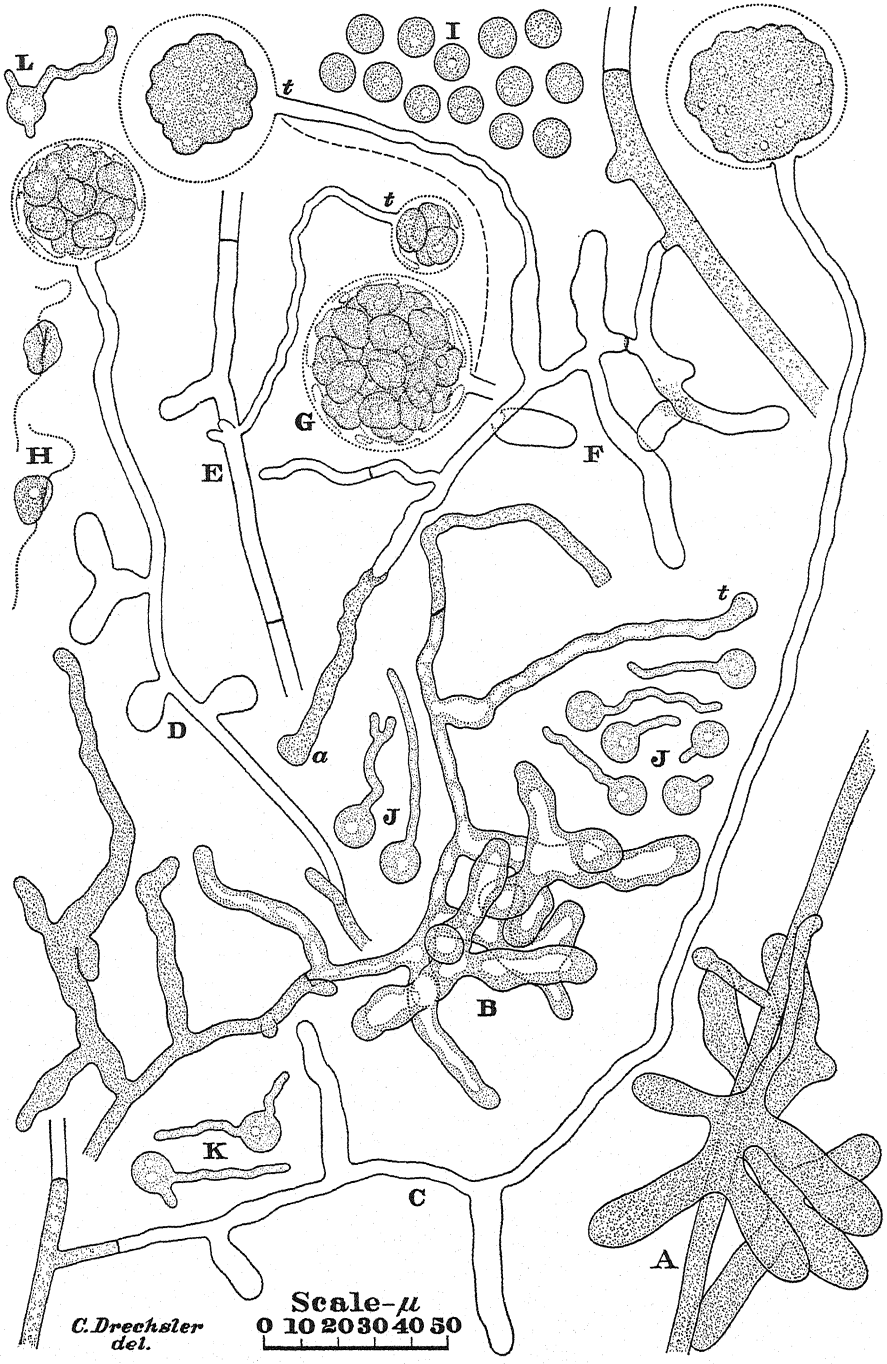



Fig. 5. Asexual reproductive apparatus of *Pythium myriotylum;* ×500 throughout. A. Hypha bearing distended sporangial branches. B. Vacuolate sporangium shortly before discharge. C. Sporangium shortly after discharge. D, E. Empty sporangia with their zoospores nearly ready to escape. F. Sporangium that, after frustration of its first evacuation tube (a), has formed a vesicle at the tip of a second tube (t). G. Same vesicle at a later stage. H. Motile zoospores. I. Encysted zoospores. J, K, L. Germinating zoospores. (t, evacuation tube.)

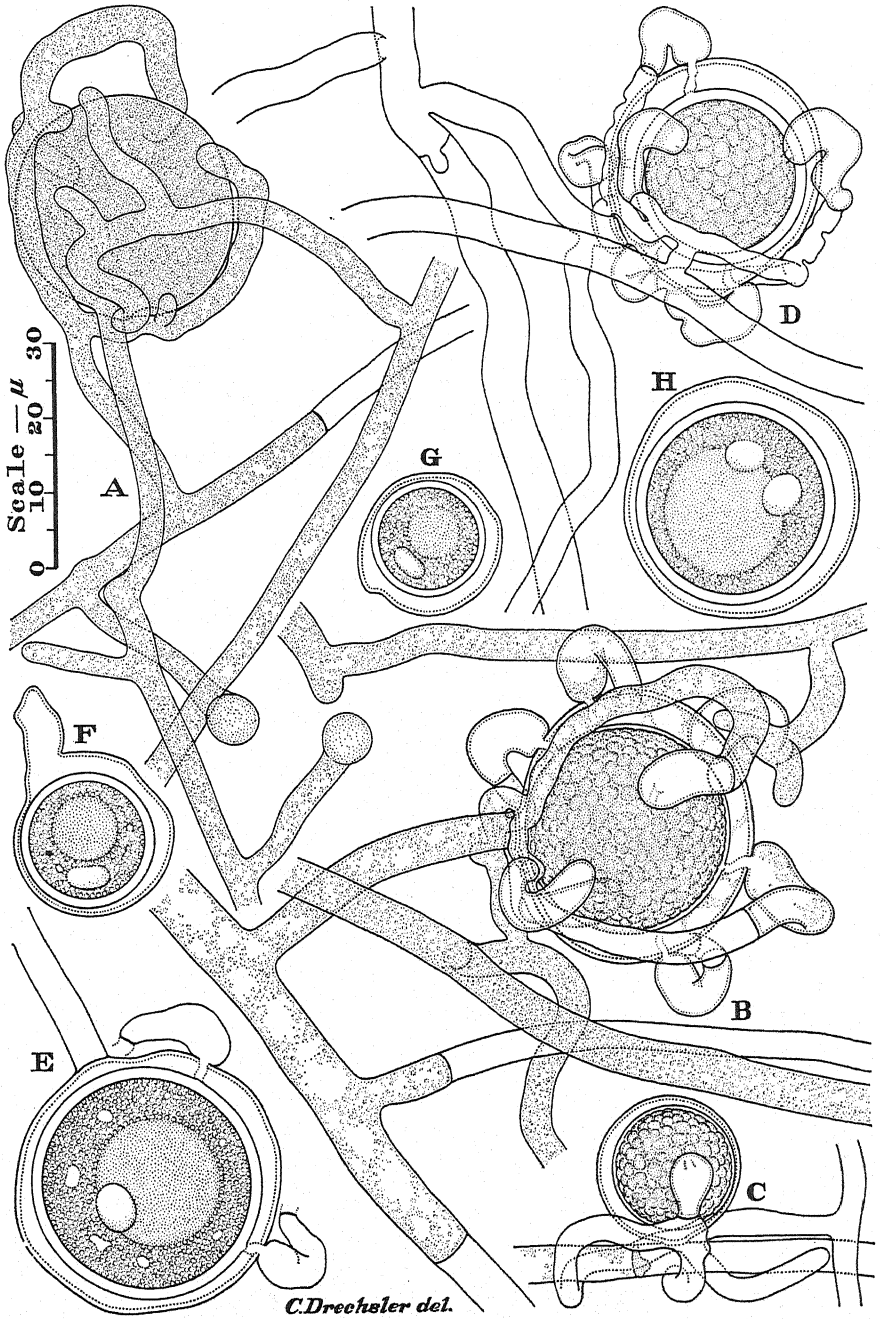



FIG. 6. Sexual reproductive apparatus of *Pythium myriotylum* formed in maize-meal-agar cultures; drawn to a uniform magnification with aid of a camera lucida; ×1000 throughout. A–D. Immature units showing antheridial relationships. E. Large oogonium with mature oospore; showing two antheridia in profile view. F–H. Oogonia, each with a mature oospore.

E, G). After increasingly violent activity the zoospores escape from the disintegrating vesicle and swim about for some time (Fig. 5, H) to round up into subspherical cysts (Fig. 5, I), which sooner or later germinate by the production of a single germ tube (Fig. 5, J), or of 2 (Fig. 5, K) or even 3 (Fig. 5, L) germ tubes.

*Pythium myriotylum,* whether growing in the tissues of host plants or in various artificial agar media, usually shows fairly abundant sexual reproduction. The earlier stages in the enlargement of the subspherical oogonium ordinarily precede development of any accompanying antheridia, though, as a rule, antheridial branches are found wrapped extensively about the expanding female organ before it attains definitive size (Fig. 6, A). The enveloping branches soon give rise terminally or, less often, somewhat laterally, to crook-necked male organs, each delimited proximally by a cross-wall. At nearly the same time the oogonium, having completed its growth, likewise is delimited from its supporting hypha. Contraction of the oogonial contents into a spherical body of lumpy structure (Fig. 6, B, C, D; Fig. 7, A, B, C) ensues, with intrusion of fertilization tubes, and movement of antheridial materials through them. The spherical body, after laying down a thick wall, undergoes internal reorganization whereby it is converted into a yellowish oospore that at early maturity (Fig. 6, E, F, G; Fig. 7, D) reveals the unitary structure most usual in oospores of the genus,—its single reserve globule being surrounded by a parietal layer of granular protoplasm, in which a single subspherical or oblate ellipsoidal refringent body is imbedded. After several weeks of further ripening, some oospores will frequently be found to contain 2 refringent bodies (Fig. 6, H; Fig. 7, E); and additional increase in number of these small but conspicuous structural elements may come with more prolonged aging. With extended aging, too, the parietal layer often acquires a more coarsely granular texture almost like the texture of the homologous layer in species of *Aphanomyces.*

The sexual apparatus of *Pythium myriotylum* shows much variety with respect to the origin and positional relationships of its component parts. Most often, perhaps, the oogonia are borne terminally on branches of variable lengths (Fig. 6, A, B, D; Fig. 7, A, B, C), yet their occurrence in lateral (Fig. 6, C; Fig. 7, E) or intercalary (Fig. 7, D) positions is not infrequent. The male complement may be supplied wholly from a single parent filament (Fig. 6, D; Fig. 7, A, B), though often 2 (Fig. 6, A, B) or 3 (Fig. 7, C) such filaments, which generally have no close mycelial connection with the oogonium, may contribute antheridial branches. Almost invariably the antheridial branches are not only wrapped about the oogonium itself, but are to some extent intricated with the filament supporting that organ; the intrication, however, being of an irregular or haphazard sort little suggestive of the symmetrical helicoid involvement characteristic of *P. helicoides* Drechsl. and *P. palingenes* Drechsl. Undersized oogonia may be supplied with only 1 (Fig. 6, C) or 2 (Fig. 7, A) antheridia, whereas larger specimens very often have 6 (Fig. 6, D), 7 (Fig. 6, B) or 8 (Fig. 7, B, C) male organs

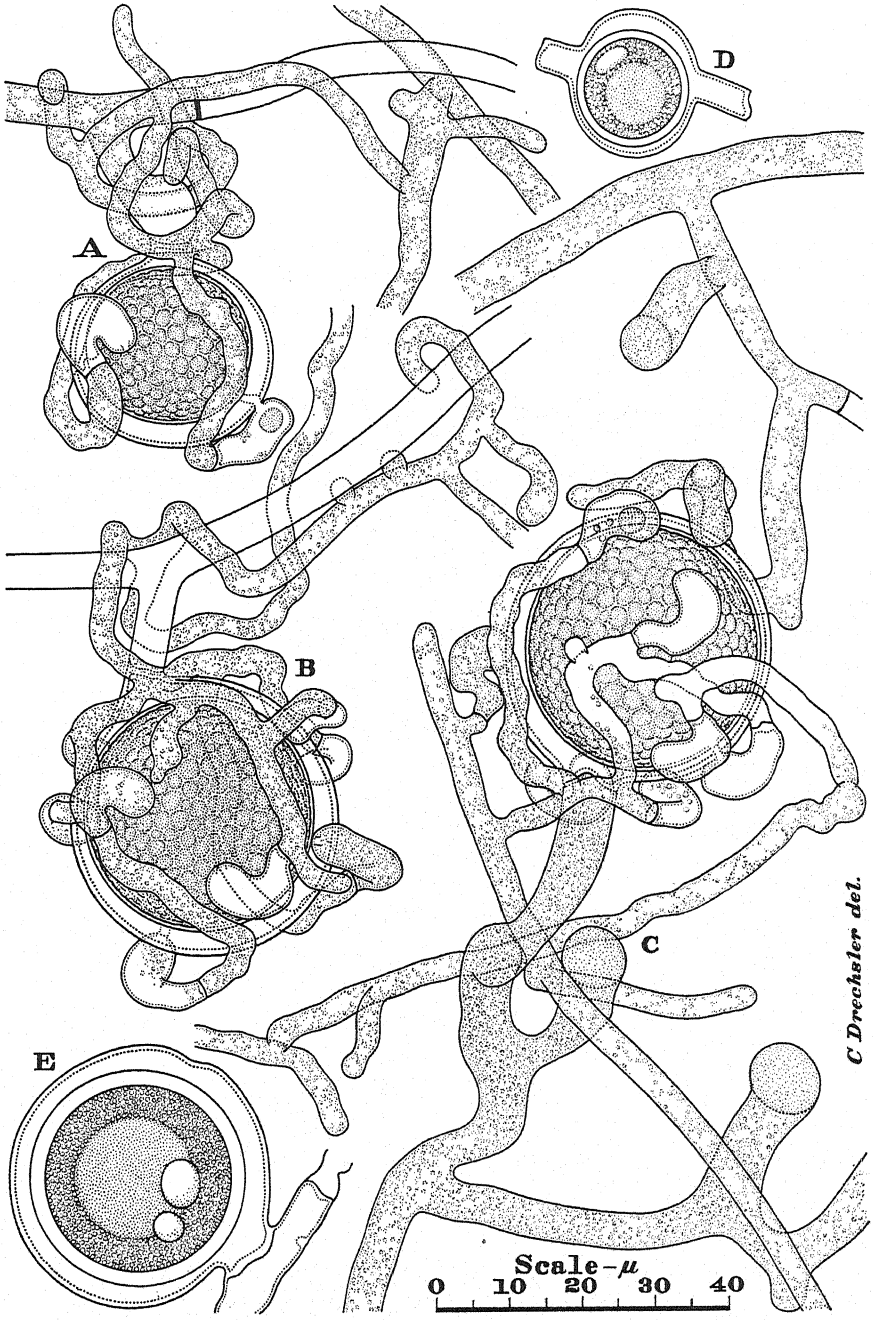



FIG. 7. Sexual reproductive apparatus of *Pythium myriotylum* formed in maize-meal-agar cultures; drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A–C. Immature units showing antheridial relationships. D, E. Oogonia, each with a mature oospore.

formed in contact with them. Somewhat comparable arrangement of sex organs and associated hyphal parts is recognizable in *P. arrhenomanes, P. graminicolum* Subr., *P. periilum* Drechsl., and *P. scleroteichum* Drechsl., despite certain readily noticeable differences pointed out earlier (17, p. 885); and it seems probable that *P. myriotylum* is more closely allied to these species than to *P. butleri,* even though the latter offers much greater similarities of parasitic habit. Analogous enwrapment of the oogonium by its male complement appears suggested also in Matthews' illustrations (26, Pl. 11, Figs, 1, 2, 3, 6) of her *P. catenulatum.*

Like *Pythium arrhenomanes* and *P. scleroteichum,* though perhaps in lesser degree, *P. myriotylum* is annoyingly given to degeneration of its sexual apparatus. Often its oogonia form oospores that degenerate before the correct unitary organization of early maturity has been achieved. Indeed, frequently degeneration sets in before even an oosphere becomes recognizable. When this happens the affected oogonium may utilize, without much evident advantage, some or all of its contents in putting forth a disorderly array of hyphal outgrowths; or, again, it may suffer spectacular invasion by a tangle of hyphae often originating from one or several of the attendant antheridia. Occasionally, the tangled mass of hyphae gives rise to a new oogonium and new antheridia, which then proceed with normal development; so that eventually an oospore of small size, yet of correct internal structure, comes into being within the old oogonial envelope.

When cultures of *Pythium myriotylum* show much degeneration they usually show also a pronounced tendency toward morphological extravagances that finds some expression even in those units of sexual apparatus that yield oospores of correct internal structure. Many of the antheridia may then be abnormally long and conspicuously upcurved or contorted, while an excessively large proportion of the oogonia and oospores may depart widely from average dimensions. Relatively large dimensions of both oogonia and oospores often accompany degeneration caused by low temperature and nutritional deficiency. Thus, when grown at 14° C. on filtered maize-meal-decoction agar the strain of *P. myriotylum* isolated from a decaying cucumber in 1924 gave 28.8 μ as the average diameter of good oospores, and 35.4 μ as the average diameter of mature oogonia. When the same strain was grown at 28° C. on maize-meal agar that contained in suspension a substantial quantity of finely divided maize-meal, and that had been slightly acidulated with hydrochloric acid, measurements on 200 sexual units selected at random gave values of 20.8 μ and 26.5 μ for average diameter of oospore and oogonium, respectively. The smaller values were mentioned in the diagnosis of the species, as they were derived from material evidently far more representative of normal development than the material that had given the larger values. For the cultures grown at the higher temperature on the more nutritious medium yielded sexual apparatus in extraordinary abundance and with virtually no degeneration; whereas, those grown at the lower temperature on the filtered medium yielded such appa-

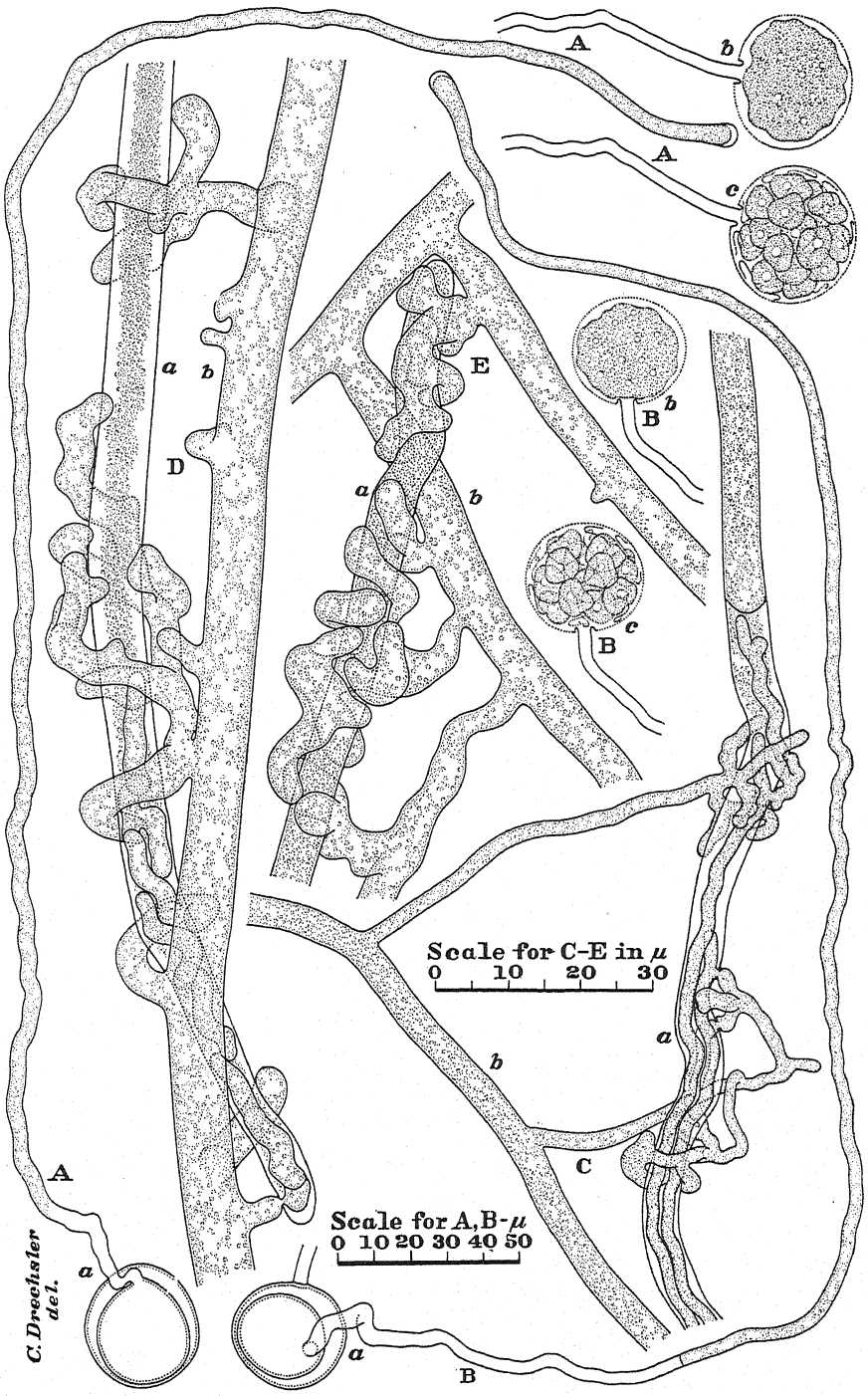



FIG. 8. A, B. Oospores of *Pythium myriotylum*, each germinating by production of a zoosporangium: a, long germ zoosporangium shortly before discharge; b, vesicle with discharged granular contents; c, vesicle with zoospores ready to escape; × 500. C. Hypha of *P. myriotylum* (a) parasitized by *P. periplocum* (b); × 1000. D. Hypha of *P. myrio-*

ratus only in moderate quantity, much of it vitiated by degeneration. Associated with the more abundant production of oogonia and oospores, and with virtual absence of degeneration, was a high degree of uniformity with respect to size. The 200 measurements of oogonial diameter from which the average of 26.5 μ was computed showed a distribution of values, expressed to the nearest integral number of microns, as follows: 16 μ, 2; 20 μ, 1; 22 μ, 1; 23 μ, 6; 24 μ, 7; 25 μ, 25; 26 μ, 54; 27 μ, 50; 28 μ, 29; 29 μ, 16; 30 μ, 5; 31 μ, 1; 32 μ, 1; 33 μ, 2; while the accompanying measurements for diameter of oospore showed the following distribution: 12 μ, 1; 15 μ, 2; 17 μ, 1; 18 μ, 6; 19 μ, 21; 20 μ, 41; 21 μ, 67; 22 μ, 37; 23 μ, 15; 24 μ, 6; 25 μ, 2; 26 μ, 1.

Oospores of correct internal structure have been found capable of germination after 2 or 3 years of storage at temperatures between 5 and 10° C. Like the reproductive bodies, sexual or asexual, of oomycetes generally, they produce vegetative germ hyphae when transferred to a fresh substratum devoid of free liquid water, or to a liquid medium containing a considerable quantity of nutrients in solution. When transferred to water containing little or no food substance they germinate more frequently by putting forth a simple filament, which in several observed instances was 500 to 600 μ long and 3 to 4 μ wide. After the protoplasmic contents have completely passed from the chamber of the oospore, and sometimes have further been evacuated from a proximal portion of the filament, the tip of the filament forms a hyaline cap of dehiscence (Fig. 8, A, a; B, a). Discharge of the germ sporangium thus brought into being proceeds as with zoosporangia of mycelial origin; the hyaline cap becoming inflated into a vesicle as it receives the migrating granular material (Fig. 8, A, b; B, b). The undifferentiated protoplasmic mass undergoes division into a swarm of laterally biciliate zoospores (Fig. 8, A, c; B, c), numbering usually 10 to 16 individuals, which escape when the vesicular membrane finally yields under the impact of their increasingly violent movements.

### A NEW PROLIFEROUS PYTHIUM OF THE HELICOIDES SERIES

In June, 1939, A. A. Dunlap of Texas Agricultural Experiment Station submitted for identification a culture of a pythiaceous fungus that, according to an accompanying letter, had been isolated from roots of wheat plants sent from the Panhandle region of Texas. Since the numerous oospores visible in the substratum were unmistakably of multiplicate internal structure the fungus was at once presumed to belong with the series of proliferous forms typified in *Pythium helicoides*. This presumption was found amply justified when the asexual reproductive stage came to light. As a member of the *helicoides* series the fungus invites attention by unusual simplicity in the make-up of its sexual apparatus.

Growing at moderate temperatures in pure culture on agar media soft enough to offer no appreciable impediment, the fungus from Texas extends its mycelium more slowly than most species of *Pythium*. A relatively rich

medium, as for example, Lima-bean agar, induces development of rather copious aerial mycelium both in Petri dishes and in test tubes. Such aerial mycelium has been observed to persist more than 2 years under fairly dry conditions, without matting down on the substratum. Some aerial growth may often be observed also when the fungus is propagated in test tubes on maize-meal-decoction agar, a medium containing nutrients in moderate or in small concentration; though in Petri dishes this substratum usually affords development only of intramatrical mycelium that presents to the naked eye a somewhat lustrous radiating appearance modified occasionally by cloud-like variegation expressive of local differences in density of texture. Under the microscope, as might be expected, the intramatrical filaments are revealed, on the one hand, as being less haphazard in their courses than the filaments making up the diffuse mycelium of *P. myriotylum,* while, on the other hand, they show less parallelism of arrangement than is evident in the conspicuously lustrous mycelia of *P. vexans* de Bary (=*P. complectens* Braun) and *P. complens.* Intramatrical hyphal branches in plate cultures ordinarily fail to form appressoria on encountering the glass floor of the Petri dish,—a failure contrasting markedly with the behavior of the closely related *P. polytylum* Drechsl. (14), which, under similar conditions, gives rise to numerous adhesive organs.

Asexual reproduction may conveniently be induced by excising sizable slabs from a Lima-bean-agar plate culture permeated with young mycelium, and transferring them to a shallow layer of sterile water in a sterilized Petri dish. At temperatures between 20 and 25° C. each irrigated slab promptly extends into the surrounding liquid a fringe of extramatrical mycelium often 5 to 10 mm. in width. Within perhaps 15 or 18 hours after the transfer some of the filaments composing the fringe give rise individually to a terminal enlargement with a papillate protrusion at its apex (Fig. 9, A). Once it attains definitive size the enlargement is delimited from the supporting filament through deposition of a basal septum (Fig. 9, B, C, D). The terminal papilla may become prolonged into an evacuation tube of somewhat variable length. Should this tube suffer functional frustration, 1 (Fig. 9, E) or 2 (Fig. 9, F) similar tubes are put forth from other positions. Sooner or later the undifferentiated protoplasmic content of the sporangium passes into a vesicle formed through inflation of a hyaline cap at the tip of the successful evacuation tube (Fig. 9, G). In this vesicle the granular protoplasm is fashioned into laterally biciliate zoospores, wholly after the manner typical of the genus *Pythium* (Fig. 9, H). Often, especially when discharge is delayed while one or more longish evacuation tubes are being put forth, a branch may grow out laterally from the supporting hypha, just below the base of the sporangium (Fig. 9, G; H; I; J, a). At other times, when discharge has been accomplished quickly by means of a short apical evacuation tube, a second sporangium may develop within the emptied envelope (Fig. 9, K), eventually to give rise to zoospores in a vesicle formed a variable distance from the orifice of the first sporangium (Fig. 9, L).

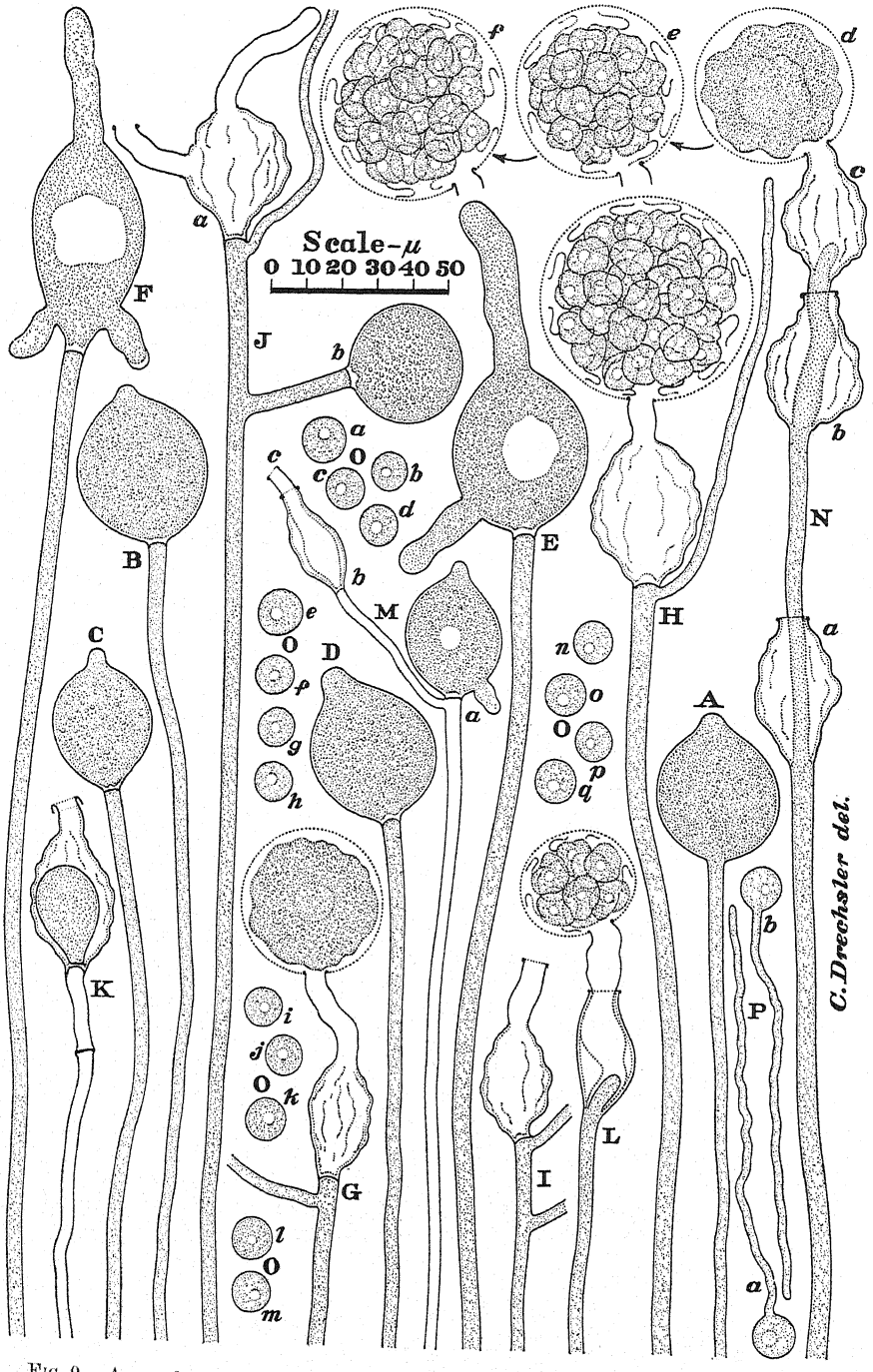



FIG. 9. Asexual reproductive apparatus of *Pythium ostracodes* produced in irrigated Lima-bean-agar preparations; drawn to a uniform magnification with the aid of a camera lucida; ×500 throughout. A. Young sporangium continuous with supporting hypha. B–D. Full-grown delimited sporangia. E, F. Sporangia with evacuation tubes. G–J. Discharged sporangia. K–M. Proliferous development of sporangia. N. Three uniaxial sporangia, (a–e); same vesicle at successive stages (d–f). O. Encysted zoospores (a–q). P. Germinating zoospores (a, b).

Owing to differences in promptness of discharge a terminal sporangium (Fig. 9, M, a) may sometimes be found still retaining its contents, even when a second and a third sporangium, formed subsequently on a lateral prolongation of the supporting filament, are present only as nested envelopes (Fig. 9, M, b, c). Such nested arrangement betokens, in the main, rather prompt discharge, as does also a seriate uniaxial arrangement of empty sporangial envelopes, brought about by repeated straightforward prolongation of the supporting filament and production of successive sporangia (Fig. 9, N, a, b, c), which, one after another, produce vesicles wherein their undifferentiated contents are transformed into zoospores (Fig. 9, N, d, e, f). After being liberated the zoospores swim about for some time before coming to rest and rounding up into subspherical cysts (Fig. 9, O, a–q) that germinate commonly by the production of a single germ hypha about 1.8 to 2 μ in width (Fig. 9, P, a, b).

Thus with respect to the morphology and development of its asexual reproductive phase, the fungus appears closely similar to the 4 proliferous species I presented earlier (14) as members of the *helicoides* series. It perhaps resembles *Pythium oedochilum* Drechsl. and *P. palingenes* more closely than *P. helicoides*, since its sporangiferous filaments, like those of the former 2 species, are little given to racemose or cymoid branching, even though now and then they may bear an auxiliary sporangium on a short branch arising some distance below the terminal sporangium (Fig. 9, J, b).

The fungus seems unusually reliable in producing normal sexual apparatus (Fig. 10, A–I; Fig. 11, A–G) on different kinds of agar culture media. On maize-meal agar containing in suspension a substantial quantity of finely divided maize-meal, its sexual development is, as a rule, not only very abundant but also to an extraordinary degree free of degeneration. The oogonium often arises as a lateral subspherical enlargement sessile on the parent filament (Fig. 10, A, B, C; Fig. 11, C); or it may be formed terminally either on a short branch (Fig. 10, D; Fig. 11, A, B), or on a longer hypha (Fig. 10, G); or, again, it may develop as a laterally intercalary (Fig. 10, E, F, I; Fig. 11, E, F) or mesially intercalary (Fig. 10, H; Fig. 11, D) body. During its earlier stages of growth it seems usually to have no direct contact with any part that can be distinguished as a male element (Fig. 10, A). On attaining definitive size it is fertilized commonly by a single antheridium (Fig. 10, B–I; Fig. 11, A–F), and only occasionally by 2 antheridia (Fig. 11, G, a, b).

Alongside of the robust oogonium the antheridium looks puny and frail, although actually it is not smaller than the corresponding organs in many of the more familiar species of *Pythium*. It consists usually of a somewhat swollen elongated cell formed terminally on a branch arising most often from the mycelial filament that either bears the oogonium directly (Fig. 10, B, C, E–I; Fig. 11, C, D, E), or gives off a short branch supporting the oogonium (Fig. 10, D; Fig. 11, A, B, G, a). In some units of monoclinous sexual apparatus, where the male branch is short and arises from a position

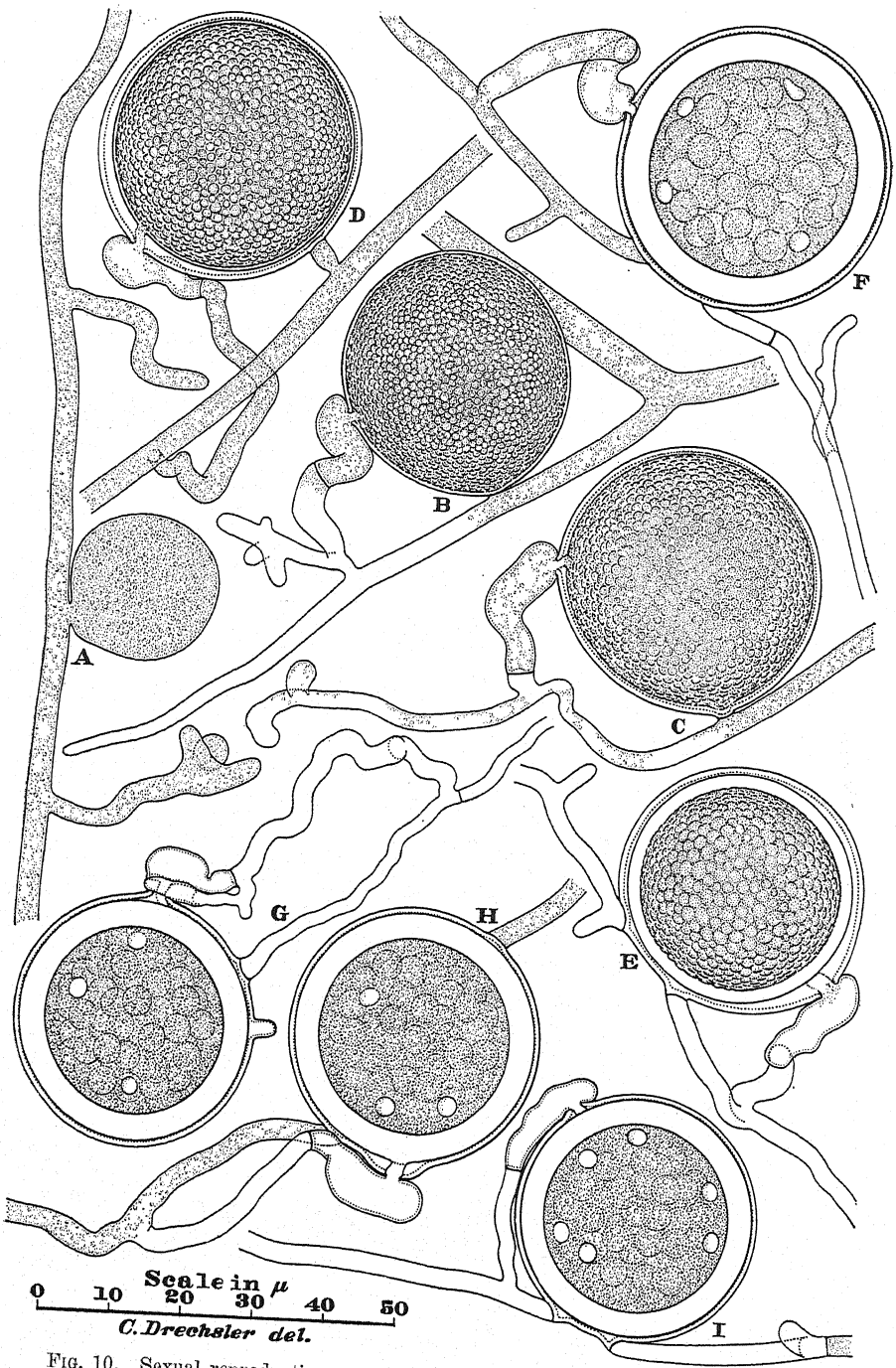



FIG. 10. Sexual reproductive apparatus of *Pythium ostracodes* formed in unfiltered maize-meal-agar medium; drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A. Hypha with a young oogonium growing out laterally. B–E. Complete sexual units in immature condition. F–I. Complete sexual units, each with its mature oospore showing multiplicate internal organization.

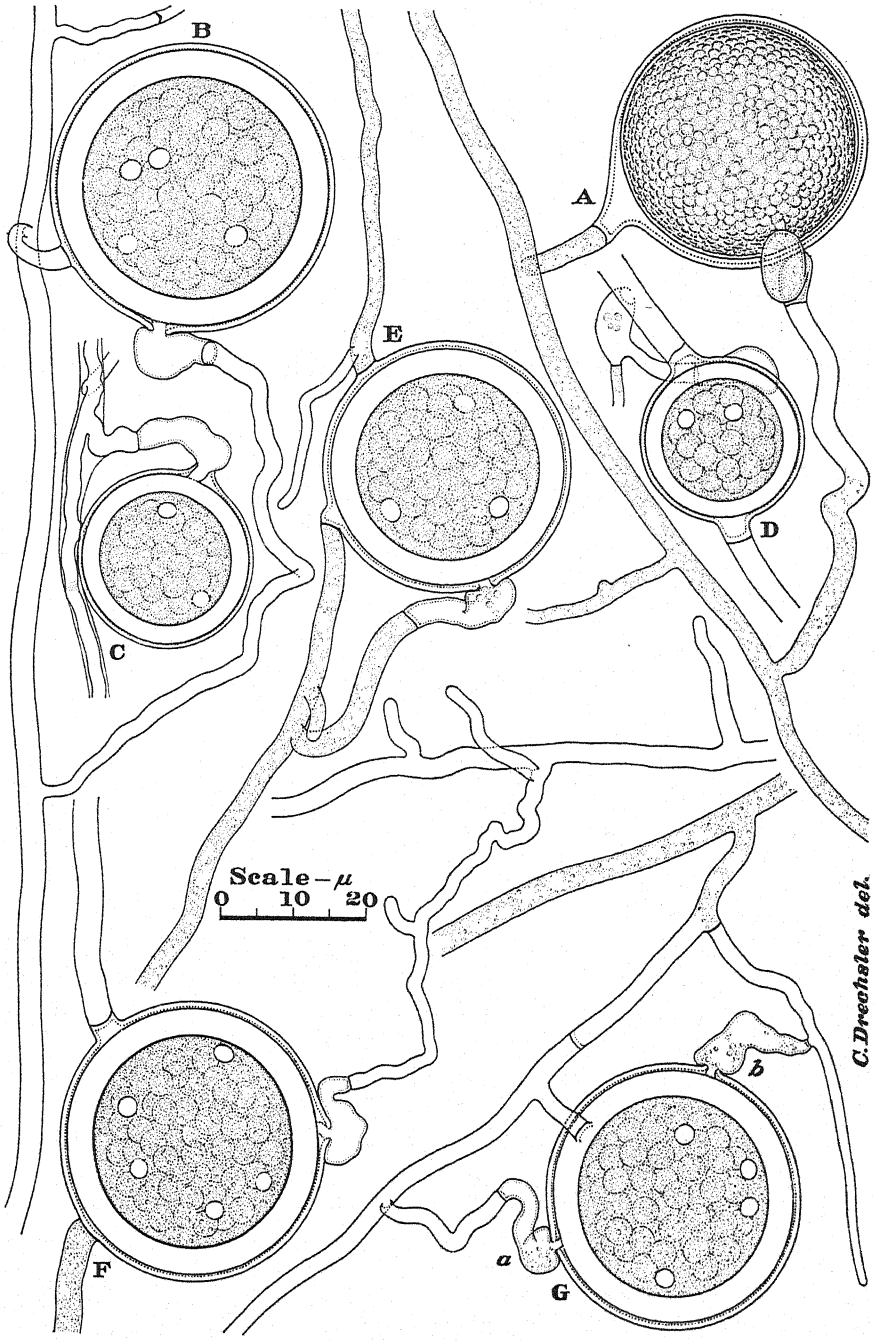



FIG. 11. Sexual reproductive apparatus of *Pythium ostracodes* formed in unfiltered maize-meal-agar medium; drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A. Complete sexual unit in immature condition. B–G. Complete sexual units, each with its mature oospore showing multiplicate internal organization; the parent hypha in C illustrating the "Durchwachsung" often found in aging material.

close to the oogonium, the mycelial parts connecting the male and female organs may have an aggregate length of only 20 μ (Fig. 10, I), while in other monoclinous units, where the male branch arises from a more distant position, the mycelial connection between oogonium and antheridium may exceed 125 μ (Fig. 11, A) or even 150 μ (Fig. 11, B) in length. In units of diclinous sexual apparatus the length of the antheridial branch is rarely less than 50 μ (Fig. 11, F), and sometimes exceeds 200 μ. Now and then an antheridium may be found attached laterally (Fig. 11, G, b),—a positional relationship that might come about either from lateral development in the beginning, or from terminal development followed by renewed elongation of the supporting branch.

The antheridium is often found applied to the oogonium laterally almost throughout its length (Fig. 10, D, H, I), much as in *Pythium helicoides*. No less often, however, it is applied laterally only along its distal portion (Fig. 10, B, E; Fig. 11, B, F); and frequently, indeed, it merely makes apical contact (Fig. 10, C, F; Fig. 11, G, a, b) in somewhat the same commonplace manner as the antheridia, for example, of *P. dissotocum* Drechsl. (21, Fig. 2, 3). Although in some instances the oogonium comes to protrude rather markedly toward the antheridium (Fig. 10, E, H; Fig. 11, C) and thus provides obvious parallelism with *P. helicoides* and *P. oedochilum*, more often the wall of the globose body bulges out only slightly where the short fertilization tube is thrust through it (Fig. 10, F, I; Fig. 11, E, G, a, b). At the time of fertilization the oogonial contents appear to consist largely of protoplasmic blocks or lumps (Fig. 10, B, C). The lumpy mass often shrinks away perceptibly from the oogonial membrane (Fig. 10, D; Fig. 11, A) before its envelopment in a thick peripheral wall of its own (Fig. 10, E) heralds the internal changes required for its conversion into an oospore of multiplicate structure (Fig. 10, F–I; Fig. 11, B–G).

Usually only 2 to 6 refringent bodies are discernible in the oospore at early maturity. This number, however, includes only the refringent bodies lying close to the spore surface nearest the observer, since those in deeper positions are at this stage rather effectively obscured by the overlying granular material and reserve globules. The reserve globules, while noticeably larger than the refringent bodies, appear smaller and correspondingly more numerous than the multiple globules in *Pythium helicoides*. As a rule they have a diameter approximately equal to the thickness of the oospore wall, which dimension is even greater here than in the 4 closely related forms previously described. The oospore usually fills the oogonial chamber so nearly completely that its distinctly yellowish wall very often appears extensively in contact with the colorless oogonial membrane; the structural separateness of the 2 envelopes then remaining recognizable chiefly because of their difference in coloration. With respect to its main dimensions the sexual apparatus ordinarily is given only to moderate variation. The relevant metric data submitted in the diagnosis below were derived from 200 measurements of oogonia selected at random in a culture showing abundant

sexual development with virtually no degeneration. These 200 oogonia gave values for diameter, expressed to the nearest integral number of microns, distributable as follows: 14 μ, 1; 18 μ, 1; 21 μ, 1; 24 μ, 1; 25 μ, 1; 26 μ, 1; 29 μ, 1; 30 μ, 1; 31 μ, 2; 32 μ, 5; 33 μ, 22; 34 μ, 21; 35 μ, 32; 36 μ, 40; 37 μ, 27; 38 μ, 17; 39 μ, 10; 40 μ, 10; 41 μ, 2; 42 μ, 2; 43 μ, 2; and the 200 oospores of correct internal structure contained within them gave measurements for diameter distributable thus: 13 μ, 1; 16 μ, 1; 19 μ, 1; 22 μ, 1; 23 μ, 1; 26 μ, 1; 27 μ, 1; 28 μ, 1; 29 μ, 3; 30 μ, 3; 31 μ, 16; 32 μ, 31; 33 μ, 25; 34 μ, 38; 35 μ, 26; 36 μ, 22; 37 μ, 11; 38 μ, 10; 39 μ, 4; 40 μ, 1; 41 μ, 2.

Maize-meal-agar cultures containing numerous oospores of correct internal organization have, on examination after 2 years of storage at temperatures between 5 and 10° C., shown relatively little degeneration among the reproductive bodies. The conspicuously thick-walled structures seem, indeed, well designed for conserving life over long periods of time. Yet despite their longevity and their strongly indurated appearance the oospores will not only germinate very promptly, but, what is perhaps more remarkable, will germinate by producing zoosporangia and zoospores in almost all instances where liquid water moderately free of nutrients is present. Thus, when the oospores produced in 0.03 cc. of unfiltered maize-meal-agar medium are distributed in 3 cc. of water over the floor of a Petri dish 90 mm. wide, motile zoospores begin to be liberated after 6 or 7 hours, and in 15 hours may usually be found swarming in spectacular abundance throughout the preparation. Such behavior suggests that under natural conditions the fungus may possibly give rise to zoospores on a greater scale in connection with the germination of its oospores than through development of sporangia from its mycelium.

The first change unmistakably associated with germination becomes evident when the oospore wall—or, rather, the thick shell-like layer over the thin membrane intimately surrounding the protoplast—shows within a region between 5 μ and 10 μ wide an array of striations extending radially from its inner contour half way toward its outer contour (Fig. 12, A). These striations signify apparently that the wall is being progressively honeycombed with radial pockets. As the striations elongate and reach the outer contour, the remnants of wall material along the inner contour vanish, leaving a space into which the protoplast and the thin pliable membrane surrounding it soon protrude (Fig. 12, B). Solution of the honey-combed fabric continues centrifugally, and soon a fairly wide channel is cleared, permitting protrusion of the protoplast to the oogonial membrane (Fig. 12, C). Meanwhile, the portion of this membrane lying in the path of the channel has become somewhat less distinct optically, and now gives an impression of reduced firmness. At all events, it yields without much resistance in allowing the protrusion to break through and to elongate externally as a stout germ tube (Fig. 12, D). Thereupon radial striations come into view everywhere along the inner contour of the thick oospore wall (Fig.

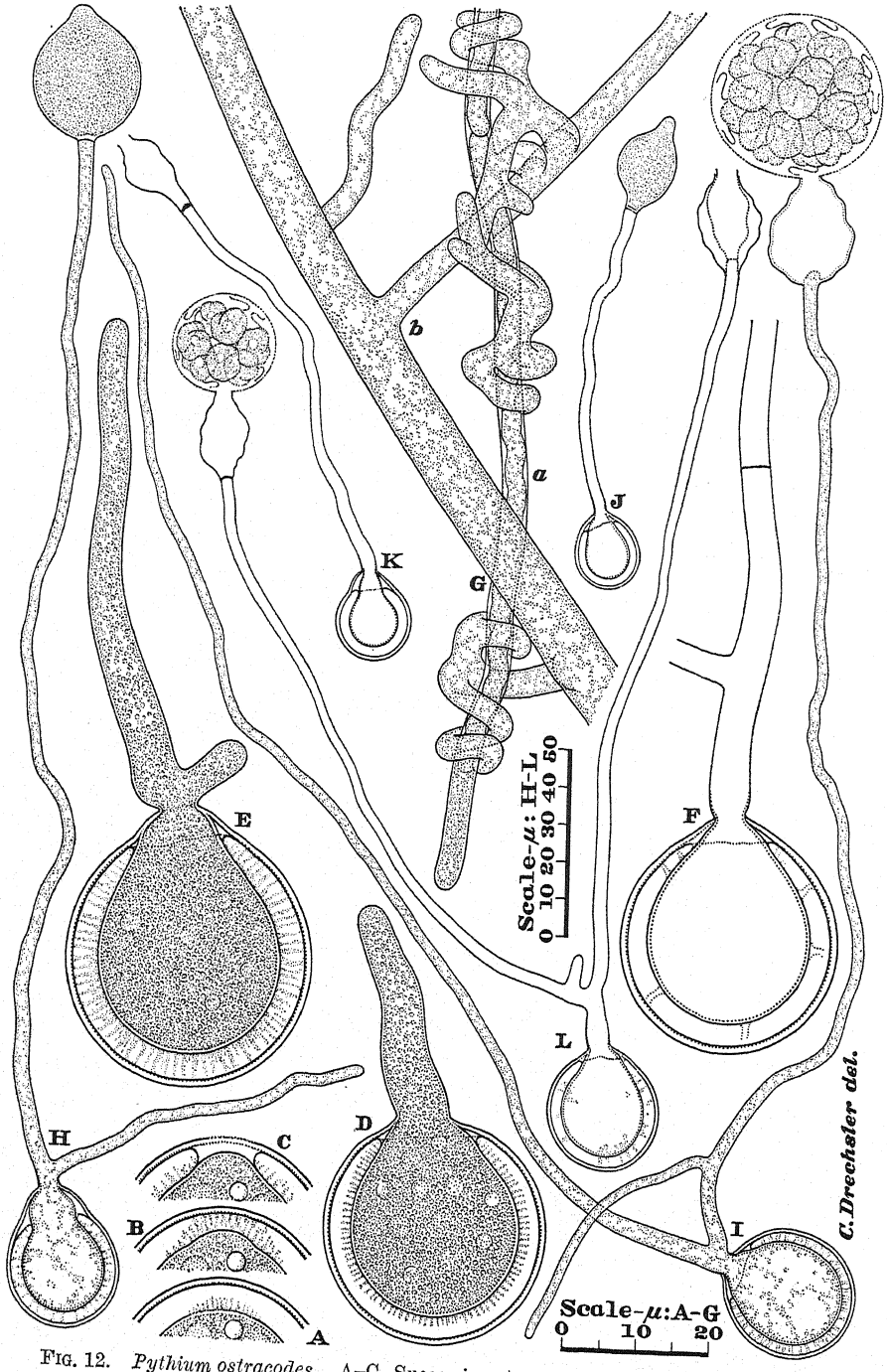



FIG. 12. *Pythium ostracodes.* A–C. Successive stages in perforation of oospore wall preparatory to germination; ×1000. D, E. Oospores with elongating germ tubes; ×1000. F. Membranous remains of oospore after germination; ×1000. G. Hypha of *P. ostracodes* (a) parasitized by spinach strain of *Aphanomyces cladogamus* (b); ×1000. H, I. Oospores which after forming one sporangium are ready to form other sporangia; ×500. J, K, L. Oospores which have exhausted their contents in forming 1, 1, and 3 zoosporangia, respectively; ×500.

12, D), and are soon extended to its outer contour (Fig. 12, E). Thus honeycombed throughout, the substance of the massive wall undergoes resorption, until finally only some few radial markings may remain as vestiges in the space between the persistent oogonial membrane and the similarly persistent membrane which intimately surrounded the protoplast (Fig. 12, F); and very often, again, even such meager vestiges are extinguished.

At about the time the first radial markings can be observed in a localized region of the massive wall surrounding them, the protoplasmic contents of the oospore begin to show significant changes. The reserve globules appear less distinct and are reduced in volume, while simultaneously the granular material is increased in corresponding measure. Temporarily the refringent bodies in the deeper portions of the protoplasmic mass become more clearly visible, so that their total number, usually between 10 and 15, and less often between 5 and 10, or between 15 and 20, can now be determined. During the period when the germ tube grows to a length of 50 to 75 μ, the refringent bodies gradually are lost to view in their densely granular matrix (Fig. 12, D, E). Usually, during this early period, too, a branch is given off by the germ tube close to its origin (Fig. 12, E). The 2 resulting elements commonly grow in rather widely divergent directions (Fig. 12, H), one or the other often putting forth an additional branch (Fig. 12, I). When a filament has attained a length of 75 to 500 μ, or sometimes even a length of 700 μ or 800 μ, it gives rise at its tip to a terminally papillate sporangium (Fig. 12, H) similar in shape to the sporangia produced from vegetative mycelium, and, like them, after development of an evacuation tube with an apex of dehiscence, discharging its contents into a vesicle for transformation into laterally biciliate zoospores (Fig. 12, I). The largest sporangia borne on germ hyphae yield between 20 and 25 zoospores, yet the production of one such organ ordinarily leaves unexpended much of the protoplasm in an oospore of good size (Fig. 12, H, I), even if some undersized oospores are completely exhausted in producing individually a short germ hypha together with a single small sporangium that may yield only between 5 and 10 zoospores (Fig. 12, J, K). Most oospores, consequently, give rise to 1 or 2 additional sporangia, in many instances with a display of proliferous development. Thus, the second sporangium often is produced on a prolongation of the filament that bore the first, while a third may be formed elsewhere at the tip of a branch (Fig. 12, L); or proliferous development may be absent, the 2 or 3 sporangia being produced on different filaments of the branching apparatus.

The more commonplace type of germination by production of mycelia ensues, of course, when oospores are transferred to agar media devoid of free water, as also when they are put into rich liquid media or into water containing substantial quantities of nutrients. In the earlier stages of such vegetative germination, digestion of the thick oospore wall proceeds much as in germination under aquatic conditions; but soon after the germ hyphae have emerged they begin to take up food substances, thereby initiat-

ing a new cycle of autonomous growth. Yet a supply of fresh water or fresh substratum is not always necessary for germination. Often, while undergoing microscopic examination, oospores have been found germinating actively within 2 hours after they had been removed, completely dormant, from maize-meal-agar cultures 6 or 8 weeks old, even though the stale ambient in which they were formed and which still surrounded them not only had received no addition of fresh water but was slowly losing water through evaporation.

As the fungus, apart from other diagnostic features, would seem distinguished from all known species of *Pythium* by its remarkably thick oospore wall, it is described as a new member of the genus under a specific name meaning "testaceous."

## Pythium ostracodes sp. nov.

Mycelium hyalinum, ramosum, in hyphis 1.8–6.5 μ crassis consistens; zoosporangiis sphaeroideis vel ellipsoideis vel citriformibus, 25–55 μ longis, 16–38 μ crassis, fere terminalibus, pluribus deinceps ex apice unius hyphae repullulantis saepe gignentibus, protoplasma eorum per tubulum in vesiculam fundentibus; tubulo 2–45 μ longo, 4–9 μ crasso, plerumque ex apice sporangii rarius ex latere ejusdem orto; vesicula 5–35 zoosporas gignente; zoosporis primo reniformibus, a latere biciliatis, mox quietis, globosis, plerumque 10–12.5 μ diam. Oogonia terminalia vel intercalaria vel lateralia, globosa, 14–43 μ (saepius circa 35.4 μ) diam., pariete 0.5–1 μ crasso circumdata; antheridiis plerumque singulis rarius binis, vulgo androgynis rarius diclinis, clavatis vel cylindratis, fere 10–26 μ longis, 4–7 μ latis, modo a fronte modo aliquatenus vel omnino a latere ad oogonium appositis, membrana saepius aliquanto undulata tectis; oosporis flavidis, sphaeralibus, vulgo 13–41 μ (saepius circa 33.5 μ) diam., muro 1.5–4.3 μ (saepius circa 3.6 μ) crasso circumdatis, 10–75 pilulas oleosas plerumque 3–4 μ crassas et 5–20 corpuscula nitida 2–3 μ crassa continentibus, prompte germinantibus denique aut mycelium emittentibus aut hypham fertilem simplicem vel saepius aliquantulum ramosam cum 1–3 zoosporangiis proferentibus.

Habitat in radicibus Tritici aestivi in Texas.

Intramatrical mycelium in transparent agar media frequently of somewhat lustrous radiate appearance, capable of approximately 15 μ radial extension in 24 hours at 24° C., composed of hyphae mostly 1.8 to 6.5 μ wide, the more delicate ramifications usually developed only in moderate quantity. Aerial mycelium sometimes absent, but at other times moderately or even rather abundantly developed, and then under conditions not too humid persisting long without collapsing.

Sporangia under aquatic conditions formed terminally on long, simple, or sparingly branched extramatrical hyphae, mostly 2.5 to 4.5 μ wide; subspherical, prolate ellipsoidal, or lemon-shaped, measuring usually from 25 to 55 μ in length and from 16 to 38 μ in transverse diameter; discharging their contents into a vesicle through an evacuation tube often somewhat reflexed, 2 to 45 μ long and 4 to 9 μ wide, usually formed as a prolongation of an apical papilla, but sometimes arising from a lateral position, especially after frustration of an apical tube; proliferous in moderate measure, either through subsporangial branching, or through uniaxial elongation of the supporting filament. Zoospores formed in numbers ranging from 5 to 35, kidney-shaped, laterally biciliate, on rounding up forming cysts mostly 10 to 12.5 μ in diameter, which germinate usually by putting forth a germ tube about 2 μ in width.

Oogonia sometimes formed terminally on lateral branches less than 25 μ in length, but more often borne directly on long filaments in terminal, lateral, laterally intercalary, and mesially intercalary positions; in some instances protruding noticeably toward the antheridium, yet generally subspherical, measuring 14 to 43 μ, mostly 30 to 41 μ (average 35.4 μ) in diameter; provided with a wall 0.5 to 1 μ in thickness. Antheridia usually occurring singly in relation to an oogonium, but sometimes 2 in number; usually formed terminally or somewhat laterally on a branch 2 to 100 μ long arising from the same hypha as the oogonium at a distance 5 to 100 μ from the oogonium, but ocasionally of diclinous origin and then usually borne terminally on a branch 50 to 250 μ long arising from a distant filament; elongated cylindrical, clavate, or saccate in shape, often with somewhat wavy contours; measuring usually 10 to 20 μ in length and 4 to 7 μ in width; sometimes applied apically, but at other times applied lengthwise for some distance below the apex, if not for the entire distance from apex to base; producing a fertilization tube often 1

to 3 μ long and 1 to 1.5 μ wide. Oospore usually distinctly yellowish, subspherical, measuring 13 to 41 μ, mostly 28 to 39 μ (average 33.5 μ) in diameter; provided with a wall 1.5 to 4.3 μ, mostly 3.2 to 4 μ (average 3.6 μ) in thickness; containing 10 to 75 reserve globules mostly 3 to 4 μ in diameter, and 5 to 20 globose refringent bodies 2 to 3 μ in diameter; germinating readily by producing a mycelium, or, under aquatic conditions, by extending a simple or somewhat branched sporangiophore, 75 to 800 μ long, whereon 1 to 3 sporangia are borne terminally, often in part through proliferous development.

Occurring in rootlets of *Triticum aestivum* in Texas.

### BIOTIC RELATIONSHIPS WITH SOME OTHER ROOT-ROTTING OOMYCETES

As might be expected, agar plate cultures that have been prepared for the isolation of root-rotting or damping-off fungi by placing pieces of affected plant material on sterile medium often afford development of mycelia belonging to 2 or 3 separate species of *Pythium*. When the several mycelia present in a culture are all referable to such familiar pathogenic species as, for example, *P. ultimum* Trow, *P. debaryanum*, *P. irregulare* Buism. and *P. mammillatum* Meurs, no hyphal relationship expressive of parasitism comes to light. When, however, a growing mycelium of one or another of these species has occasion to meet a growing mycelium of *P. oligandrum* Drechsl. (14), a species with echinulate oogonia, which occurs rather frequently in diseased underground parts of many phanerogamic plants, the former is attacked by the latter in a violently parasitic manner. Everywhere in the region of encounter delicate ramifications of the spiny form enwrap the filaments of the other elaborately, soon penetrating in different places and intruding hyphal processes that grow lengthwise through the filaments to assimilate the degenerating host materials. Here and there the internal hyphae give off branches that push their way out through the enveloping host membrane and, after some elongation, bring about the destruction of other host filaments nearby. During their earlier stages of development oogonia, as well as conidia or zoosporangia, may be invaded, though oospores appear immune from attack once they are surrounded by their thick wall. Frequently the attack of *P. oligandrum* on the congeneric forms commonly associated with damping-off is so devastating that in extensive tracts of substratum they can give rise to only a few reproductive bodies.

The same destructive parasitism came to light again and again when *Pythium oligandrum* was intentionally planted in dual culture with the familiar pathogenic forms found attacked in isolation cultures. Among more than a score of additional congeneric species that were likewise grown in dual culture with *P. oligandrum*, a few were parasitized with similar violence. Most of the others, including *P. myriotylum*, suffered less severe, though readily appreciable, injury. The remaining forms, of which *P. ostracodes* may serve as a convenient example, incurred little or no injury, even though in some instances their hyphae were intricately enveloped by branches of the spiny form. *P. complens* at times attacked *P. oligandrum* on a small scale.

As *Pythium acanthicum* and *P. periplocum* Drechsl. in their morphology show close kinship with *P. oligandrum*, these two species, better known

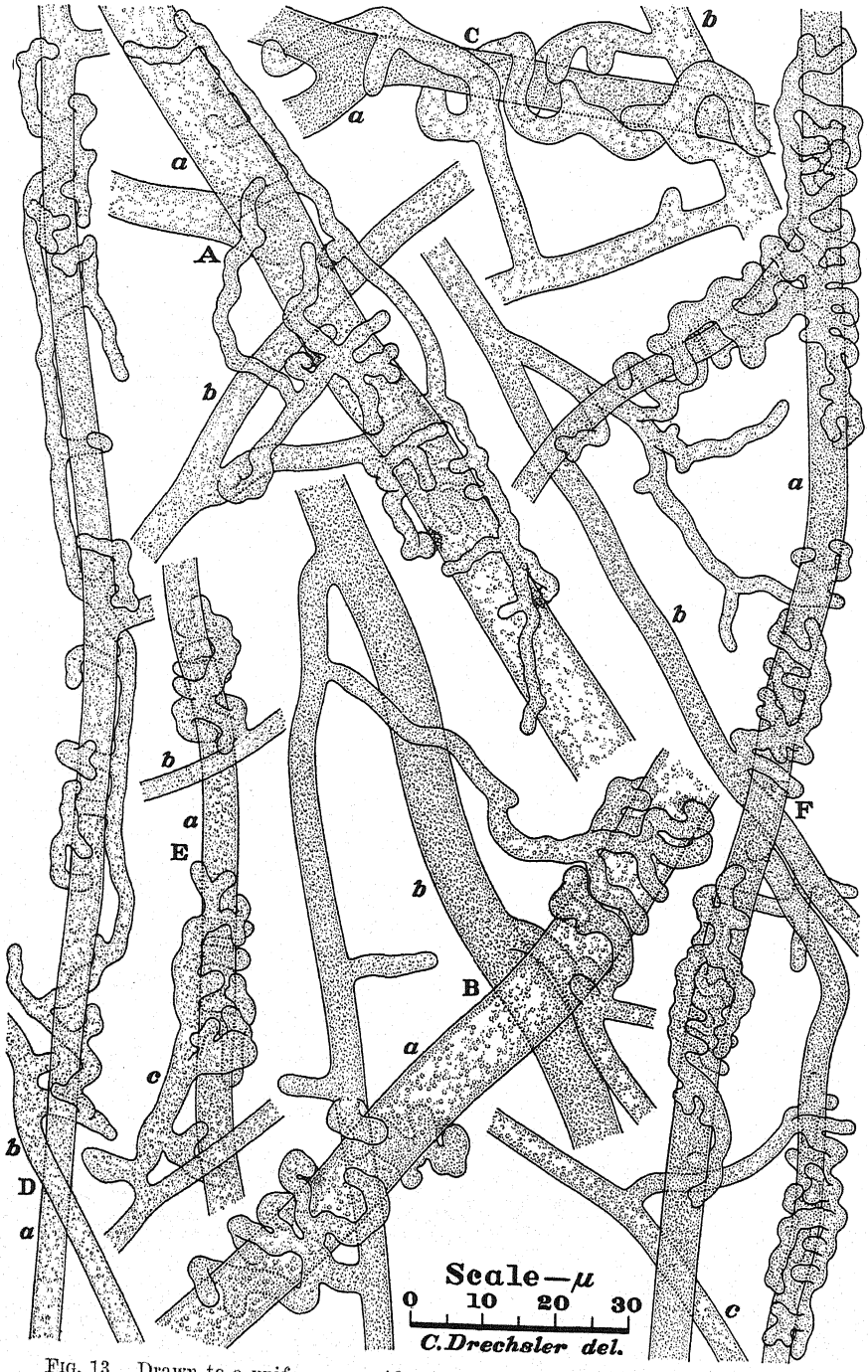



FIG. 13. Drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A. Hypha of *Pythium myriotylum* (a) attacked by *P. acanthicum* (b). B. Hypha of *P. myriotylum* (a) attacked by *P. oligandrum* (b). C. Hypha of *P. myriotylum* (a) attacked by spinach strain of *Aphanomyces cladogamus* (b). D. Hypha of *P. ostracodes* (a) scantily enwrapped by *P. periplocum* (b). E. Hypha of *P. ostracodes* (a) more densely enwrapped by *P. periplocum* (b, c). F. Hypha of *P. ostracodes* (a) enwrapped by *P. oligandrum* (b, c).

to me from their causal connection with blossom-end rot of watermelon than from their association with root decay, were also grown in series of dual cultures with a wide assortment of congeneric forms. In the 2 series of cultures parasitism and antagonism of varying degrees of severity again prevailed; the performance of the fruit-rotting fungi being generally similar to that of *P. oligandrum*. *Pythiogeton autossytum* Drechsl. (15), a member of the Pythiaceae, though alien to the genus *Pythium*, was attacked unmistakably. However, when the 3 echinulate forms were grown in dual culture with a number of saprolegniaceous forms occurring in association with root rot of crop plants, they not only proved innocuous, but like numerous other species of *Pythium* tried out, were themselves subjected to adverse action. In the case of *Aphanomyces cochlioides* Drechsl. (13) this action appeared to be mainly defensive, and caused little positive injury. Elaborate enwrapment of the pythiaceous filaments marked the more aggressive and injurious attack of *Plectospira myriandra* Drechsl. (11) as well as of the 3 strains of *Aphanomyces*, referable apparently to *A. cladogamus* Drechsl. (13) that were isolated, respectively, from diseased roots of pansies, *Viola tricolor* L. (16), spinach, *Spinacia oleracea* L. (18), and flax, *Linum usitatissimum* L. (18).

*Pythium myriotylum*, as has been intimated, is attacked by *P. acanthicum* (Fig. 13, A; Fig. 14, A), *P. periplocum* (Fig. 8, C; Fig. 14, B), and *P. oligandrum* (Fig. 13, B; Fig. 14, C, D) less severely than *P. ultimum* or *P. debaryanum*. On encountering its mycelium the spiny forms put forth numerous delicate branches that make contact with the filaments of *P. myriotylum*, and often enwrap them more or less extensively. Some of the delicate branches become slightly enlarged at the tip, which then is applied closely to the wall of the *myriotylum* filament, where often it is visibly cemented in place by means of an opaque secretion. The terminations thus present the appearance of appressoria, and, indeed, sometimes operate as such by intruding haustorial threads into the filament to which they are applied (Fig. 8, C; Fig. 14, D). These haustorial threads grow lengthwise within the invaded filament until their further extension in either direction is checked by a septum that the host has in the meantime laid down at some distance from the place of ingress. Since the granular contents of the host disappear locally as invasion proceeds, it may be presumed that they are assimilated by the haustorial threads, and that, accordingly, the relationship present here is an unambiguously parasitic one. Yet, in most cultures the greater proportion of *myriotylum* hyphae invested by branches of the spiny forms are not actually invaded. Often these hyphae show abnormal modification both in somewhat increased thickness of the peripheral wall generally and in much more pronounced local thickening on the inner side of the wall at places where appressoria have become attached. The protoplasm within them often remains in normal condition for a protracted period, but often, too, it migrates to other portions of the mycelium, or undergoes gradual degeneration. Similar migration and

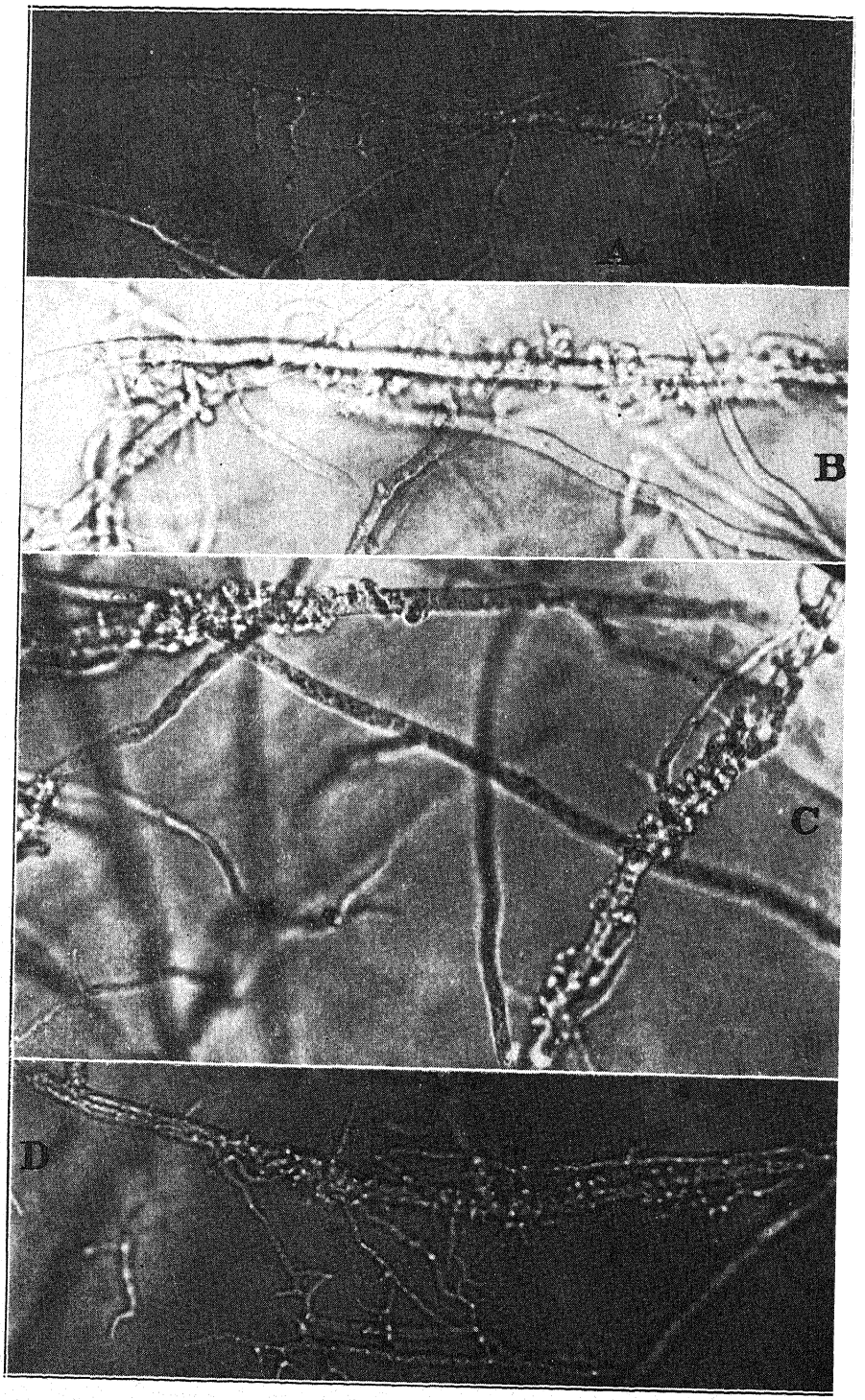



FIG. 14. A. Hypha of *Pythium myriotylum* attacked by *P. acanthicum*. B. Hyphae of *P. myriotylum* attacked by *P. periplocum*. C, D. Hyphae of *P. myriotylum* attacked by *P. oligandrum*.

degeneration of protoplasm takes place in some measure also in vigorous mycelium of *P. myriotylum* that, after being removed to a glass slide, is subjected to microscopical examination; so that the harmful effect of the enveloping branches is not easily ascertainable. Nevertheless the impression usually is gained that the branches are injurious, at least locally. It is not obvious, however, that the spiny forms obtain food materials from the *myriotylum* filaments merely intricated by them, unless, perchance, after the intricated filaments have been made to degenerate internally, their soluble contents may become available in part by diffusing out into the ambient. The expanded terminations that sometimes serve as appressoria seem hardly large enough to operate effectively as cupping organs.

When a growing mycelium of *Pythium myriotylum* encounters a growing mycelium of *Plectospira myriandra* (Fig. 8, E) or meets a growing mycelium of the pansy (Fig. 8, D), the spinach (Fig. 13, C), or the flax strain of *Aphanomyces cladogamus,* its hyphae in the zone of encounter are promptly enveloped in an elaborate manner by short ramifying branches, which, except for their greater width, resemble the branches extended under similar conditions by the 3 echinulate species of *Pythium.* As this envelopment proceeds the protoplasmic contents of the affected hyphae lose their normal appearance and become more or less opaque (Fig. 8, E; Fig. 13, C). Unmistakable disorganization promptly follows. Sometimes the enveloped portions of hyphae are, in addition, invaded internally (Fig. 8, D), but the injury accruing locally to the mycelium of *P. myriotylum* is obvious even where no invasion occurs. In any case the enveloped portions of hyphae, whether undergoing destruction through parasitism or through antagonism, are soon delimited from the adjacent healthy portions by cross walls.

Although *Pythium myriotylum* thus sustains some evident injury on encountering antagonistic oomycetous forms, its general development in dual culture is often impaired less than that of the fungus inflicting the injury. Under certain conditions of culture *P. acanthicum* and the strains of *Aphanomyces cladogamus* from pansies, spinach, and flax, are unable to advance any considerable distance beyond the zone of encounter into the tract of substratum already permeated with mycelium of *P. myriotylum;* whereas *P. myriotylum* will sooner or later spread over the tract of substratum originally occupied only by the opponent fungus. Under similar condition of culture, *Pythium periplocum* and *Pythium oligandrum,* as well as *Plectospira myriandra,* usually will halt the mycelial advance of *P. myriotylum* abruptly at the zone of encounter, while continuing their own advance into the tract already occupied by *P. myriotylum.* In such advance, however, their envelopment and destruction of hyphae diminish markedly, whether because they are adversely affected by the increasing staleness of the substratum, or because the older hyphae of *P. myriotylum* are less subject to attack. Meanwhile, as the older mycelium of the opponent species loses in vegetative vigor through aging and onset of sexual repro-

duction, the mycelium of *P. myriotylum* often recovers initiative and resumes growth by advancing in thinner array beyond the zone of encounter; so that, eventually, its oospores, like those of the opponent species, may often be found distributed in all portions of the culture. The ability of *P. myriotylum* to offset the discomfiture suffered in the zone of encounter seems attributable in part to its more prolonged vegetative period and its ready production of aerial mycelium.

When a growing mycelium of *Pythium ostracodes* (Fig. 15, A, a) encounters a growing mycelium of *P. acanthicum, P. periplocum* (Fig. 15, A, b) or *P. oligandrum* in a Petri-plate culture, both usually continue to advance without interference becoming manifest macroscopically in modification of their circular outlines. Indeed, on microscopical examination, sometimes only a few hyphae of *P. ostracodes* may be found enveloped by branches of the opposed spiny form. At other times, however, especially when a rather soft agar medium has been used in the preparation of the dual culture, numerous hyphae of *P. ostracodes* in the zone of encounter are very elaborately enveloped in ramifications of *P. periplocum* (Fig. 13, D, E), of *P. oligandrum* (Fig. 13, F; Fig. 16, A), or of *P. acanthicum.* On either side of the zone of encounter much less envelopment is noticeable; the intrication present in the tract where new hyphae from one of the 3 echinulate species have encroached upon aging hyphae of *P. ostracodes* usually being somewhat more abundant than that present in the tract where new hyphae of *P. ostracodes* have encroached upon old hyphae of the spiny form. It is remarkable that however elaborately the hyphae of *P. ostracodes* may be enveloped by any one of the 3 echinulate species, they commonly show no injury from such envelopment.

A far different result is obtained when *Pythium ostracodes* (Fig. 15, B, a) is grown in dual culture with *Plectospira myriandra* (Fig. 15, B, c; Fig. 16, B) or with any one of the strains of *Aphanomyces cladogamus* isolated from diseased roots of spinach (Fig. 12, G), flax, and pansies. In such dual culture the mycelial advance of *P. ostracodes* is abruptly halted wherever it encounters the mycelium of the saprolegniaceous form, which continues to grow with unabated rapidity. In the zone of encounter and soon afterwards also in tracts closer to their origin the hyphae of *P. ostracodes* are elaborately enveloped by branches of the opponent fungus, and their protoplasmic contents made to degenerate (Fig. 16, B). Here and there a filament may suffer internal invasion (Fig. 12, G). The injury thus sustained by the *Pythium* mycelium often results in noticeable reduction in the quantity of oospores formed by it.

Evidence indicating some sort of biotic relationship between oomycetes associated with damping-off and other diseases destructive to terrestrial phanerogamic plants was brought forward by de Bary (2) more than 60 years ago. In the garden cress (*Lepidium sativum* L.) seedlings that he used for substratum, this investigator never observed his *Pythium artotrogus* to occur alone: in all instances he found it accompanied by a congeneric

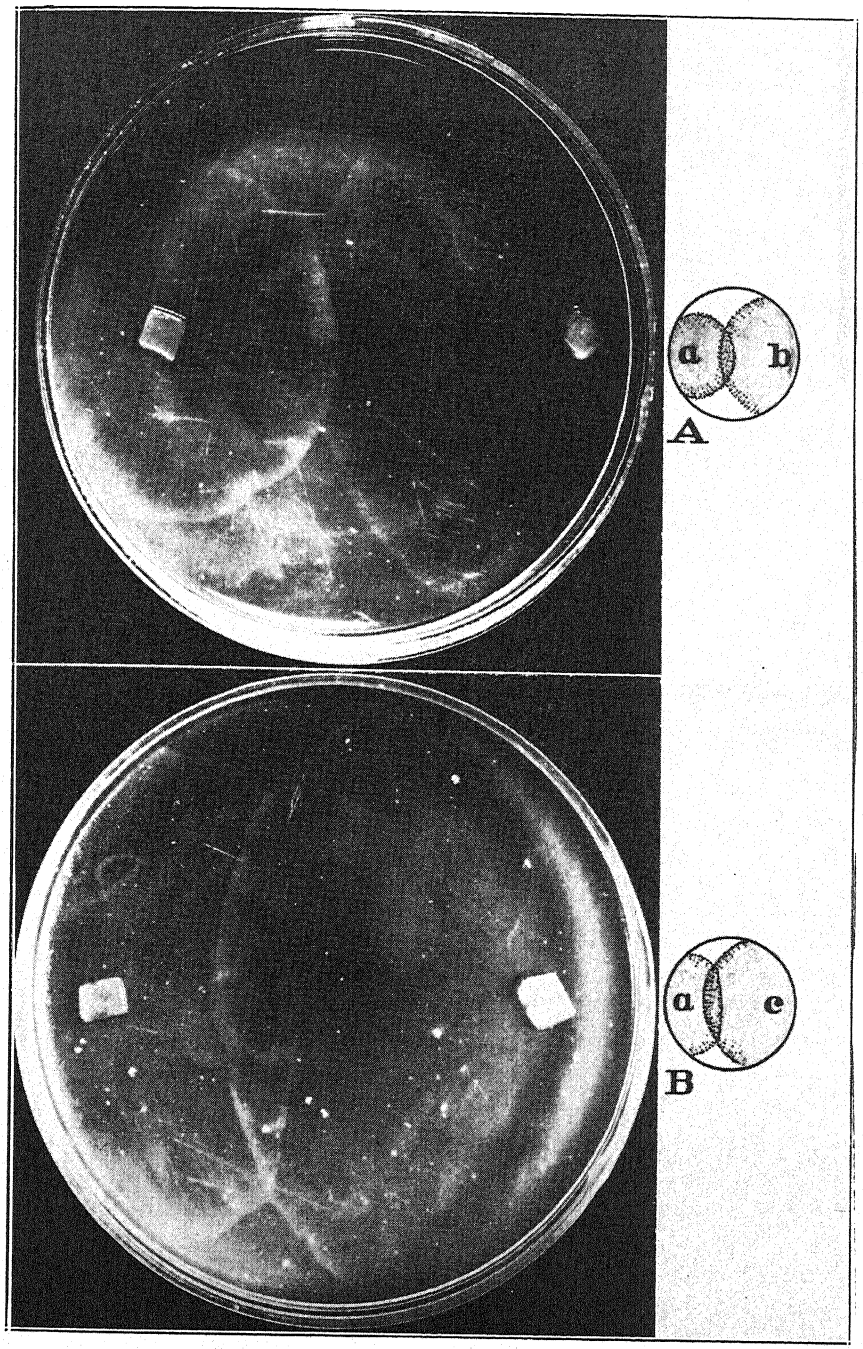



FIG. 15. Growing mycelium of *Pythium ostracodes* (a) encountering growing mycelium of *P. periplocum* (b), the rounded outlines of both mycelia showing no sign of retarded advance. B. Mycelium of *P. ostracodes* (a) encountering growing mycelium of *Plectospira myriandra* (c); the former, as is manifest from the lesser convexity of the portion of its outline within the region of encounter, being retarded or halted in its advance.

FIG. 16. A. Mycelium of *Pythium ostracodes* attacked by *P. oligandrum*. B. Hyphae of *P. ostracodes* attacked by *Plectospira myriandra*. The enveloped hyphae in A have remained normal, whereas those in B have collapsed.

species, which he identified as the widely pathogenic *P. debaryanum.* The congeneric species always appeared first, or at least became recognizable first through its earlier formation of reproductive organs. De Bary made repeated attempts to propagate *P. artotrogus* separately by removing living pieces of germ hyphae from its germinating oospores and placing them in drops of water to which were then added pieces of living and of scalded cress tissue. Though the germ hyphae branched abundantly in the liquid of the cultures thus prepared, the ramifying filaments consistently failed to infect either the living or the scalded tissue fragments, and consequently perished after a few days. Yet, in similar cultures to which *P. artotrogus* had been added, together with the related fungus recognized as *P. debaryanum,* the former always reproduced, even if at times only meagerly, by giving rise to its distinctive spiny oogonia.

De Bary offered 2 alternative explanations for the curious behavior of *Pythium artotrogus:* the fungus might require substances for growth that are absent in living or scalded cress tissue, but become available when such tissue is acted on by some other fungus, as, for example, *Pythium debaryanum;* or it might subsist as a parasite on *P. debaryanum,* much as species of *Chaetocladium* or *Piptocephalis* subsist as parasites on Mucoraceae. Further, de Bary had noted association of *P. artotrogus* with other fungi in its occurrence elsewhere than on cress seedlings in the laboratory. Thus, he had observed it associated with *Phytophthora infestans* (Mont.) de Bary in brown lesions on potato (*Solanum tuberosum* L.) stems, and with *P. infestans* as well as with *Pythium vexans* in dead tissue of potato vines. Again, in dried potato material in an authentic specimen of Montagne's *Artotrogus hydnosporus*—which species he held identical with his own *P. artotrogus*—he had observed spiny oogonia associated with swollen bodies of some alien fungus that might possibly have represented conidia of *P. debaryanum.*

De Bary's observations on *Pythium artotrogus* have been given some corroboration in several later writings. Butler's (4) report that he found *P. artotrogus* in rotting potato tubers attacked by *Phytophthora infestans* in Calcutta in 1902 tends to support the presumption of a habitual biotic association; though his further statement that for some time he successfully maintained the spiny fungus in cultures apparently free of *Phytophthora* hyphae, as also Mitra's (28) subsequent report that *P. artotrogus* had been found in stored potatoes in India, growing individually or associated with *P. infestans,* would seem at variance with the presumption of an obligate association. Under the binomial *Pythium hydnosporum* (Mont.) Schroet. supposedly the same spiny fungus was reported by Clinton (5) in Connecticut to have been observed not only in rotten potato tubers that had first been injured by *P. infestans,* but also in pea (*Pisum sativum* L.) roots injured by *Phytophthora cactorum* (Leb. and Cohn) Schroet., and especially abundantly in rotting grapes injured by the black-rot fungus and the grape berry moth. Sawada (31), working in Formosa, found *P. hydno-*

*sporum* in old tissue in a portion of a leaf of *Boehmeria nivea* Hook. and Arn. that had been attacked by *P. cactorum*. Dissmann (8), in Bohemia, while studying the relationship of premature decay of water lily (*Nymphaea candida* Presl.) foliage to the development, more particularly, of the 2 *Pythium* species he designated as *P. undulatum* Pet. and *P. proliferum* de Bary, noted that *P. artotrogus* sometimes occurred concomitantly with these 2 species, though only in older disintegrating portions of affected leaves,—a manner of occurrence he set forth as illustrating de Bary's view that the spiny form is adapted to the utilization of nutrient products supplied or modified by other fungi.

There is reason to suspect that among these additional first-hand reports on the occurrence of *Pythium artotrogus* in association with other fungi, as, indeed, also among the several first-hand reports mentioning its occurrence regardless of any such association, some may have been based on material not truly referable to de Bary's species. It is not evident that the different observers, with the exception of Butler (4), gave sufficient heed to the necessity for ascertaining whether their fungi showed correspondence with de Bary's (1) description in regard more particularly to fertilization of the oogonium by an antheridium consisting always of an adjacent hyphal segment, usually not modified externally, about 3 to 6 times longer than wide, and delimited by deposition of a septum in the supporting filament. Where determinations were based only on similarities to the oogonium of *P. artotrogus*—which organ was described originally (2) as being mostly 18 to 27 μ in diameter, and as being beset with tapering spines 3 to 6 μ long—the specimens might readily have belonged, instead, to *P. acanthicum*, *P. periplocum*, or *P. oligandrum*. In the morphology of their oogonia my 3 echinulate species show such close agreement with the illustrations and description of *P. artotrogus* that, from the beginning, I considered them intimately akin to de Bary's fungus. The agreement in morphology of oogonium would now seem to be sustained in parallelism of biotic relationship. This parallelism, it is true, would seem relieved by some noteworthy divergence. Contrary to de Bary's experience with *P. artotrogus*, my 3 species grow and reproduce well on many kinds of substrata, both natural and artificial, without the intervention of any other fungus; and 2 of them, moreover, are known to flourish under natural conditions as autonomous parasites on at least one phanerogamic host.

Attack on other phycomycetes by a saprolegniaceous fungus solely through external enwrapment of their hyphae was set forth by Couch (6) in the original descriptive account of his *Aphanomyces exoparasiticus*. Among the phycomycetes found subject to envelopment and injury was an unnamed species of *Pythium*. Although in one instance a direct protoplasmic connection was seen between a filament of this *Pythium* and a branch of *A. exoparasiticus*, no interchange of materials was observed. Apparently, *A. exoparasiticus* never invades the hyphae that it has enveloped, and that, as a result of being enveloped, have suffered disorganization

of their protoplasmic contents; so that, like *A. cochlioides* in some combinations, it would seem to lack even such scant internal assimilative apparatus as is formed under analogous conditions by *Plectospira myriandra* and by the 3 strains of *A. cladogamus* isolated from diseased roots of pansies, flax, and spinach. Unless the externally applied ramifications are capable of serving far better as cupping structures than appearances suggest, the elaborate attack of the several saprolegniaceous root-rotting forms here discussed would seem directed more to thwarting the development of competing fungi than to utilizing their substance as nourishment. In fine, the aggressive relationships displayed by these forms would seem to hold a larger measure of antagonism than of parasitism.

## SUMMARY

Under warm, moist conditions, *Pythium myriotylum* produces abundant aerial mycelium, which, on encountering solid objects, gives rise to innumerable appressoria in dense clusters. This manner of growth makes possible the aerial parasitism whereby the fungus is enabled, much like *P. butleri,* to destroy bulked vegetables such as cucumbers, summer squash, and string beans. *P. myriotylum* rather closely resembles *P. butleri* also with respect to its asexual reproductive stage, though noticeable differences are present in the less pronounced inflation of its zoosporangial branches and in its somewhat less bountiful production of zoospores. Its sexual reproductive stage, however, differs greatly from that of *P. butleri,* seeming, rather, to betray intimate taxonomic kinship with *P. arrhenomanes.* The oospores, of unitary internal organization, often germinate, when bathed in fresh water, by putting forth a long simple germ hypha, which, after having received the entire protoplasmic contents, dehisces terminally as a zoosporangium.

A species of *Pythium,* isolated in Texas from wheat roots, is described as new under the binomial *P. ostracodes.* Its production of subspherical zoosporangia at the tips of hyphae, together with its development of oospores having multiplicate internal organization, characterizes it as a member of the *helicoides* series. In this series it is distinguished more especially by the very simple make-up of its sexual apparatus; fertilization being accomplished usually by a single antheridium borne terminally on a branch arising from the same hypha that supports the oogonium either directly or on a short stalk. When bathed in fresh water its oospores germinate very readily by producing, often in part through proliferous development, 1 to 3 terminal subspherical zoosporangia. During germination the very thick, shell-like, colored, outer layer of the oospore wall becomes conspicuously honeycombed with radial pockets and canals as its substance undergoes progressive resorption.

*Pythium myriotylum,* like numerous congeneric forms, is attacked by *P. acanthicum, P. periplocum,* and *P. oligandrum,* though less destructively than the familiar pathogenic species *P. ultimum, P. debaryanum,* and *P.*

*irregulare*. In their attack the 3 echinulate fungi send out numerous branches that invest the *myriotylum* hyphae, in some places merely bringing about noticeable abnormality, in some other places inducing local disorganization of protoplasmic contents, and in still other places intruding assimilative elements internally. The 3 echinulate species often envelop filaments of *P. ostracodes* more elaborately without, however, causing any appreciable injury. Further, the hyphae of *P. myriotylum* and *P. ostracodes*, as well as of many other species of *Pythium*, are subject to elaborate envelopment also by such saprolegniaceous root-rotting forms as *Plectospira myriandra* and the 3 strains of *Aphanomyces cladogamus* occurring in diseased roots of pansies, flax, and spinach, respectively. Invasion sometimes follows envelopment, but even when invasion does not occur the enveloped *Pythium* filaments suffer local disorganization of their contents. The seemingly meager utilization of the degenerating materials suggests that the relationship may be antagonistic more largely than parasitic.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY,
U. S. HORTICULTURAL STATION,
BELTSVILLE, MARYLAND.

## LITERATURE CITED

1. BARY, A. DE. Untersuchungen über die Peronosporeen und Saprolegnieen und die Grundlagen eines natürlichen Systems der Pilze. Abhandl. Senckenb. Naturf. Gesell. 12: 225–370. 1881.
2. ———. Zur Kenntnis der Peronosporeen. Bot. Ztg. 39: 521–530, 537–544, 553–563, 569–578, 585–595, 601–609, 617–625. 1881.
3. BRIQUET, J., *et al.* International rules of botanical nomenclature. Ed. 3. Gustav Fischer (Jena). 1935.
4. BUTLER, E. J. An account of the genus *Pythium* and some *Chytridiaceae*. India Dept. Agr. Mem. Bot. Ser. 1, (5). 1907.
5. CLINTON, G. P. New or unusual plant injuries and diseases found in Connecticut, 1916–1919. Conn. Agr. Exp. Stat. Bull. 222: 397–482. 1920.
6. COUCH, J. N. Notes on the genus *Aphanomyces*, with a description of a new semi-parasitic species. Jour. Elisha Mitchell Sci. Soc. 41: 213–227. 1926.
7. DEIGHTON, F. C. Mycological work. Ann. Rept. Dept. Agr. Sierra Leone 1935: 22–26. 1936.
8. DISSMANN, E. Vergleichende Studien zur Biologie und Systematik zweier *Pythium*-Arten. Arch. Protistenk. 60: 142–192. 1927.
9. DRECHSLER, C. The cottony leak of cucumbers caused by *Pythium aphanidermatum*. Jour. Agr. Res. [U. S.] 30: 1035–1042. 1925.
10. ———. The cottony leak of eggplant fruit caused by *Pythium aphanidermatum*. Phytopath. 16: 47–49. 1926.
11. ———. Two water molds causing tomato rootlet injury. Jour. Agr. Res. [U. S.] 34: 287–296. 1927.
12. ———. *Pythium arrhenomanes* n. sp., a parasite causing maize root rot. Phytopath. 18: 873–875. 1928.
13. ———. The beet water mold and several related root parasites. Jour. Agr. Res. [U. S.] 38: 309–361. 1929.
14. ———. Some new species of *Pythium*. Jour. Wash. [D. C.] Acad. Sci. 20: 398–418. 1930.
15. ———. A species of *Pythiogeton* isolated from decaying leaf-sheaths of the common cat-tail. Jour. Wash. [D. C.] Acad. Sci. 22: 421–449. 1932.
16. ———. Vascular wilt and root rot of pansies due to *Aphanomyces* sp. (Abstract) Phytopath. 24: 7–8. 1934.
17. ———. *Pythium scleroteichum*, n. sp. causing mottle necrosis of sweet potatoes. Jour. Agr. Res. [U. S.] 49: 881–890. 1934.

18. ————. Occurrence of a species of *Aphanomyces* on roots of spinach and flax. (Abstract) Phytopath. 25: 14–15. 1935.
19. ————. Several species of *Pythium* causing blossom-end rot of watermelons. Phytopath. 29: 391–422. 1939.
20. ————. Three species of *Pythium* with large oogonial protuberances. Phytopath. 29: 1005–1031. 1939.
21. ————. Three species of *Pythium* associated with root rots. Phytopath. 30: 189–213. 1940.
22. ————. Three species of *Pythium* with proliferous sporangia. Phytopath. 31: 478–507. 1941.
23. HALL, C. J. J. VAN. Ziekten en plagen der cultuurgewassen in Nederlandsch-Indië in 1924. Meded. Inst. Plziekt. (Batavia) 67: 1–53. 1925.
24. HARTER, L. L., and W. A. WHITNEY. A transit disease of snap beans caused by *Pythium aphanidermatum*. Jour. Agr. Res. [U. S.] 34: 443–447. 1927.
25. JOCHEMS, S. C. J. Parasitaire stengelverbranding bij Deli-tabak. Meded. Deli Proefst. Medan Sumatra, ser. 2, 49: 35 pp. 1927.
26. MATTHEWS, VELMA D. Studies on the genus *Pythium*. 136 pp. The University of North Carolina Press (Chapel Hill). 1931.
27. MEURS, A. Parasitic stemburn of Deli tobacco. Phytopath. Zeitschr. 7: 169–185. 1934.
28. MITRA, M. Report of the Imperial Mycologist. Sci. Rept. Agr. Res. Inst. (Pusa) 1924–1925: 45–57. 1925.
29. PARK, M. Report on the work of the mycological division. Admin. Rept. Dir. Agr. Ceylon 1936: D28–D35. 1937.
30. RANDS, R. D., and E. DOPP. Variability in *Pythium arrhenomanes* in relation to root rot of sugarcane and corn. Jour. Agr. Res. [U. S.] 49: 189–221. 1934.
31. SAWADA, K. Descriptive catalogue of the Formosan fungi, Part III. Dept. Agr. Gov. Res. Inst. Formosa, Japan, Rept. 27. 1927.
32. SIDERIS, C. P. Taxonomic studies in the family Pythiaceae I. *Nematosporangium*. Mycologia 23: 252–295. 1931.
33. UPPAL, B. N. Soft rot of ginger. Ann. Rept. Dept. Agr. Bombay 1938–39: 206. 1940.
34. WAGER, V. A. Descriptions of the South African Pythiaceae, with records of their occurrence. Bothalia 4: 3–35. 1940.
35. WALKER, M. N. Investigations of and control of fungous diseases of watermelons. Ann. Rept. Fla. Agr. Exp. Stat. 1939–1940: 107–108. [1941].

## CORRECTIONS

In my paper entitled "Three species of *Pythium* with proliferous sporangia" (Phytopath. 31: 478–507. 1941) the line preceding the last line on page 504, and reading, "sporangia, which often show proliferous development. In an old culture on" should properly follow the second line of text on page 505. A somewhat less bewildering error in line 20 on page 499 of the same paper may be corrected by changing the word "furthering" to "further."—C. D.

# STUDIES ON GENOTYPES OF TOBACCO RESISTANT TO THE COMMON-MOSAIC VIRUS[1]

H. H. McKinney[2]

(Accepted for publication August 24, 1942)

## INTRODUCTION

Resistance to common mosaic caused by Nicotiana virus 1 is manifested in several degrees in *Nicotiana tabacum* L. Clayton, Smith, and Foster (1) recognized three classes of resistance in terms of symptoms. Class 1 denotes none to very few markings; class 2, distinct diffuse spotting; and class 3, mild systemic mottling. A more detailed understanding of some of these classes, especially class 1, is desired from the standpoints of tobacco improvement, virus synthesis, and resistance. This report covers the results of a rather general survey to determine differences in the amounts of virus produced in some of the typical genotypes, and effects of age of plant on virus synthesis and movement. The studies were conducted at Arlington Farm, Virginia. A brief abstract of the results has been published (4).

## FIELD STUDIES

### Materials and Methods

Several resistant and two susceptible tobaccos were studied, but most of the work was done with (1) a selection of Ambalema; (2) T.I. No. 448, Sel. A; and (3) Wisconsin-Havana Seed. The selection of Ambalema used seems to be one of the most resistant yet isolated from the collections obtained by Nolla and Roque (8). This selection when infected usually manifests a few diffuse light-green spots on the leaves of young plants when grown in the field. In the greenhouse these spots occur less frequently. Mosaic mottling never occurs. T.I. 448 was collected in the vicinity of Mt. Tolima, Colombia, S. A. This area is not far from the area where Ambalema tobacco was collected in the Department of Valle del Cauca (8). Morphologically, the two collections used are nearly identical; they mature late and produce as many as 50 or more dark-green leaves. T.I. 448A has never exhibited mosaic mottling. Among more than 250 plants inoculated with the virus of common mosaic, only 12 manifested faint, light-green, diffuse zones on 1 to 3 leaves per plant. These were so inconspicuous that they would not be observed except with special care. These zones were not more frequent in the field than in the greenhouse. Wisconsin-Havana Seed tobacco is very susceptible to common mosaic, has a midseason-maturing habit, and produces 40 or more dark-green leaves.

[1] Studies supported in part by funds provided under Bankhead-Jones Project S.R.F. 2–17, U. S. Department of Agriculture, Bureau of Plant Industry and Bureau of Agricultural Chemistry and Engineering cooperating.

[2] Senior pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The writer wishes to acknowledge the assistance of Matthew Koerner in conducting the tests, and also the aid provided by E. E. Clayton of the Division of Tobacco Investigations in the matter of field facilities and plant material.

In 1937, a number of resistant collections were grown in the field. As soon as the plants were well established, after transplanting, they were inoculated with Nicotiana virus 1 by wiping the leaves. In late July and early August the plants were 6½ to 7½ feet tall. At that time, the three classes of resistance mentioned above were plainly evident. On August 3, ten plants from each of several collections within each class were individually assayed. The assay samples, two leaves (6 to 10 inches long) near the top of each plant were placed in tight screw-top bottles, brought to the laboratory immediately, and frozen. The procedures followed in obtaining the extracts and making the assays are described in the chapter on greenhouse studies.

In 1938, field-grown plants were studied as in 1937, except that the 10-plant samples were batched, and the upper and middle leaves were assayed separately. The plants had set many seed pods, and all leaves on the main stalk were fully developed.

## Results

In tables 1 and 2 it will be noted that a total of 8 collections failed to manifest visible signs of infection. Three of these, T.I. 448A, T.I. 450, and

TABLE 1.—*Summary of assays for presence or absence of Nicotiana virus 1 in upper leaves of resistant classes of Nicotiana tabacum grown in the field in 1937, samples collected August 3. Ten plants of each plot were tested*

| Variety or T.I. No. | Symptoms July 23 and Aug. 3 | Lesions per bean plant from non-diluted juice of each tobacco plant | Tobacco plants with upper leaves free of virus |
|---|---|---|---|
| | | *Number* | *Per cent* |
| 448A | None | 0 | 100 |
| 450 | do | 0 | 100 |
| 320 | do | 0 to 12 | 40 |
| 384A | do | 0 to 27 | 20 |
| Ambalema | do | 0 to 100 | 10 |
| 439A | Severe diffuse spotting | 100 to 550[a] | 0 |
| 329 | Mild mottling | 100 to 550[a] | 0 |

[a] Estimates based on sample counts.

T.I. 1110B, yielded no virus in the upper leaves late in the season, whereas Ambalema, T.I. 320, T.I. 384A, T.I. 470A, and T.I. 472A3, had virus in similar leaves in some of the plants.

In table 2 it will be observed that T.I. 448A was the only one that failed to yield virus from the leaf samples collected midway between the bases and tops of the plants late in the season. Of the resistant types, Ambalema carried the greatest amount of virus in these leaves.

The types manifesting severe diffuse spotting (T.I. 439A) and mild mottling (T.I. 329) in table 1, and the very susceptible variety, Maryland Medium broadleaf, in table 2 carried relatively large amounts of virus.

TABLE 2.—*Summary of assays for presence or absence of Nicotiana virus 1 in upper and in middle leaves of mature plants in resistant classes and in a susceptible variety of tobacco grown in the field in 1938*

| Variety or T.I. No. | Symptoms Aug. 20 and 28 | Lesions per bean plant from upper leaves of 10 tobacco plants. Collected Aug. 20. No dilution | Lesions per bean plant from middle leaves of 10 tobacco plants. Collected Aug. 28. Diluted 20 ×[a] |
|---|---|---|---|
| | | *Number* | *Number* |
| 448A | None | 0.0 | 0.0 |
| 1110B | do | 0.0 | 4.5 |
| 470A | do | 0.6 | 3.9 |
| 472A3 | do | 2.0 | 0.1 |
| Ambalema | do | 3.0 | 140.0 |
| Maryland Medium | Severe mottling | 102.2 | 4.9[a] |

[a] The extract from Maryland Medium was diluted $2 \times 10^{-4}$ times in buffer. With a dilution of 20 × the lesions would be too numerous to count.

## GREENHOUSE STUDIES

### Materials and Methods

The plants were grown in earthen pots and transplanted to pots of increasing size, up to 8 inches in diameter, as their development required. Nicotiana virus 1 was used in all tests. Greenhouse temperatures were near 21° to 24° C., except when the solar energy was high. During the summer the glass was covered with a light sprayed mixture of cement, whiting, glue, and water. Temperatures were not controlled. Solutions of calcium nitrate were added to the soil at suitable intervals.

Leaf samples for assay were removed from well-watered plants that had been in the laboratory until turgid or when limp leaves were collected, they were placed with their cut ends in water in the laboratory and allowed to stand until turgid. This procedure tended to reduce some of the error in obtaining the tissue extracts. When small samples were studied, the fresh tissue was weighed and placed in a mortar with a small amount of phosphate buffer at pH 7.0. After some reduction, a little more buffer was added. Except when stated otherwise, this process was repeated until 3 parts of buffer had been added. The mortaring continued until the tissue had been reduced to very fine particles. The resulting soupy mass was poured into a double-cheesecloth-lined funnel and the liquid collected in a test tube. The extract was used for inoculations with or without further dilutions, depending on requirements. The assays were made on the primary leaves of bean plants held near 33.5° C. for 40 to 64 hours after inoculation (6).

Thorough grinding of the tissues is necessary to free the traces of virus frequently encountered in the resistant varieties. However, the method has been found to be the best yet devised for studying small samples of highly susceptible tissues as practically all virus is freed into the excess of liquid.

Of the several test plants studied, *Phaseolis vulgaris* L. (variety Scotia),

and *Nicotiana glutinosa* L., proved the most sensitive for detecting traces of virus, but in the case of the bean, satisfactory results depend to a large extent on the high culture temperature following inoculation.

When large samples were studied the fresh tissue was cut in very small pieces, placed in tightly stoppered bottles and kept frozen until tested. For testing, the tissue was pressed under hydraulic pressure and the extract was used, with or without further dilution, to inoculate the leaves of the test plants.

## Experimental

*Concentration of Virus in Leaves.*—To determine the relative amount of virus recoverable from the top, center and lower leaves of T.I. 448A, Ambalema, and Wisconsin-Havana Seed, the young plants were inoculated April 1.

On May 30, leaves were removed from the bases, middle portions, and near the tops of 4 plants of each of the three types.

Plants of T.I. 448A and Ambalema ranged from 48 to 52 inches tall with no floral buds in evidence, and the plants of Wisconsin-Havana Seed ranged from 36 to 42 inches tall and were just beginning to bloom.

The topmost leaves sampled were 3 to 4 inches long; these, with 2 leaves directly below, made up the top-leaf sample. One middle leaf and a basal leaf were used from each plant. The basal leaves were just beginning to show the first senile yellowing and none of these had been wiped with virus. All midribs were discarded from the leaf tissue.

The corresponding samples from each of the 4 plants within each type were batched. All samples were finely cut with a sharp knife, placed in tight bottles, and frozen. On June 9, the samples were extracted, diluted in buffer, and inoculated on the primary leaves of Scotia bean plants. Owing to the large number of samples, only those dilutions were assayed that previous preliminary tests indicated would give the essential information on each plant type and leaf location. On June 15 another assay was made. The results are assembled in table 3.

These data indicate that the top leaves of T.I. 448A and Ambalema manifest less virus activity than the lower leaves, and that the virus content of T.I. 448A was less than that of Ambalema. As in the field material, the T.I. 448A plants in this test failed to yield detectable virus in the upper leaves.

In Wisconsin-Havana Seed, the first assay indicated the greatest amount of virus in the top leaves. However, the second assay indicated the greatest amount in the bottom leaves. Other tests indicate that the lower leaves of this variety yield the most active virus, but it appears that the difference is not always great.

*Virus Increase in T.I. 448A.*—To determine if virus can increase in the leaves of half-grown and of nearly mature healthy plants, and to obtain information on related phases, several experiments were conducted. In

TABLE 3.—*Results of assays for Nicotiana virus 1 in leaves from upper, middle and lower portions of stems of T.I. 448A, Ambalema and Wisconsin-Havana Seed tobacco. Tests conducted during spring and summer months, 1938*

| Tobacco variety or T.I. number | Number of lesions per bean plant at indicated dilutions from— | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Top leaves, diluted— | | | | | Middle leaves, diluted— | | | | | Basal leaves, diluted— | | | | |
| | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ |
| 448A | | | | | | | | | | | | | | | |
| 1st assay[a] | 0.0 | ..... | ..... | ..... | ..... | 8.0 | 0.6 | 0.2 | ..... | ..... | 498 | 305 | 72 | ..... | ..... |
| 2nd do [b] | ..... | ..... | ..... | ..... | ..... | 0.9 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 0.2 |
| Ambalema | | | | | | | | | | | | | | | |
| 1st assay | 4.0 | ..... | ..... | ..... | ..... | 355.0 | 221.0 | 67.0 | ..... | ..... | ..... | 534 | 239 | ..... | ..... |
| 2nd do | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 0.3 | ..... | ..... | ..... | ..... | 1.8 |
| Wisconsin-Havana Seed | | | | | | | | | | | | | | | |
| 1st assay | 779.0 | 825 | 467 | ..... | ..... | ..... | ..... | 400.0 | 114 | ..... | ..... | ..... | 317 | 110 | ..... |
| 2nd do | ..... | ..... | ..... | ..... | 1.5 | ..... | ..... | ..... | ..... | 1 | ..... | ..... | ..... | ..... | 12.0 |

[a] Data in first assay were obtained on 5 plants (10 leaves) of Scotia bean plants for each diluted sample.

[b] Data in second assay were obtained on 35 half leaves of 70 Scotia bean plants. The samples were divided into two groups of 3 and 4 samples, respectively, and each sample within a group occurred on each of the 35 plants.

some cases comparisons were made with leaves of healthy Ambalema and Wisconsin-Havana Seed. Tests were made during the summer.

A nondiluted native extract of virus was wiped on 19 well-developed leaves of a single plant of T.I. 448A, and 12 to 19 days later each inoculated leaf was extracted, diluted to 3 volumes in buffered solution and wiped on 5 bean plants. Beginning with the lowest leaf position on the tobacco plant and proceeding upward, the 19 leaves induced lesions per bean plant, respectively, as follows: 22, 69, 84, 84, 33, 63, 29, 48, 14, 7, 11, 10, 59, 5, 9, 7, 23, 16, and 2. A few of the lesion counts cited are very low. It is possible that some of these lesions were from virus adhering to the tobacco leaves after wiping and do not represent virus increase. However, leaves were rinsed very thoroughly after wiping.

Very little detectable virus adheres to tobacco leaves when they are properly rinsed. Such leaves have been extracted immediately after being inocu-

TABLE 4.—*Relative amounts of virus detectable in the inoculated leaves of three types of tobacco when all leaves were inoculated as the plants were nearing maturity. Data in terms of lesions per bean plant. Extracts diluted $10^{-1}$ with buffer*

| Leaf groups | Leaves in group | Lesions per bean plant from | | |
|---|---|---|---|---|
| | | T.I. 448A | Ambalema | Wisconsin-Havana Seed |
| | *No.* | *No.* | *No.* | *No.* |
| Upper | 4 | 0.0 | 0.8 | 265.0[a] |
| Upper middle | 6 | 0.6 | 0.5 | 283.0 |
| Lower middle | 6 | 4.7 | 11.1 | 381.0 |
| Lower | 6 | 16.5 | 6.0 | 136.0 |

[a] Because of the relatively low dilution, these counts are proportionally low in comparison with those from T.I. 448A and Ambalema.

lated and rinsed, and the extracts wiped on Scotia beans. Usually no lesions appear, but occasionally as many as 3 have been observed on a leaf.

In a second experiment, similar inoculations were made on the upper, virus-free leaves of nearly mature plants of T.I. 448A that had virus in the lower leaves. All the inoculated upper leaves gave evidence of supporting virus increase of the order shown in the above test on those plants that were virus-free throughout before the inoculation. Thus it seems evident that virus present in the lower leaves did not induce resistance in the upper leaves of T.I. 448A.

In a third experiment, T.I. 448A was compared with Ambalema and Wisconsin-Havana Seed. The plants were virus-free and were approaching the flowering stage. Beginning with upper leaves, which were 3 inches long, all leaves were wiped with a fresh native extract. Thirteen days later they were collected in groups of 4 to 6 leaves each and assayed on bean leaves. The number of lesions per bean plant induced by the extracts from the several leaf groups are assembled in table 4.

The fact that the extracts were diluted $10^{-1}$ in this test may explain the absence of detectable virus in the upper leaves of T.I. 448A. However, the

lower virus activity in leaves of the upper groups is evident, especially in the resistant types. The drop in activity in the lower leaves from Ambalema and Wisconsin-Havana Seed may be due to the fact that these leaves became senile earlier than the lower leaves of T.I. 448A.

Leaves 3 to 6 inches long at the top of vegetating tobacco plants are difficult to wet because of the excessive waxy exudate and the compactness of the trichomes. This may explain some of the reduction in lesion counts on beans inoculated from these leaves. These factors, however, would not involve more than the top 3 or 4 leaves wiped. The topmost group of leaves on the Wisconsin-Havana Seed produced much virus (Table 4).

*Progress of Virus in the Plant.*—To determine the progress of virus in T.I. 448A and Ambalema when inoculated in an advanced stage of development, a number of tests were made in comparison with Wisconsin-Havana

TABLE 5.—*Progress of Nicotiana virus 1 in large plants of T.I. 448A and Ambalema tobacco as evidenced by the number of lesions per bean plant induced by nondiluted leaf extract from selected tobacco leaves above and below the zones of inoculation. Assays were made 48 days after inoculation. Samples diluted in 3 parts of buffered solution*

| Leaf positions[a] | Lesions per bean plant from virus from | | | | | | |
|---|---|---|---|---|---|---|---|
| | T.I. 448A, plant No. | | | | Ambalema, plant No. | | |
| | I | II | III | IV | I | II | III |
| 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0 | 11 | 0 |
| 22[b] | 6 | 2 | 10 | 3 | 0 | 33 | 7 |
| 16 | 1 | 0 | 0 | 0 | 0 | 8 | 11 |
| 12 | | | | | | | |

[a] Leaf positions are listed by leaf number, counting from the base of the plant, at time of assay.
[b] Inoculated leaves.

Seed. The plants were 3.5 to 4 feet tall at the time of inoculation. Fresh native virus extract was applied by wiping 3 leaves 6 to 10 inches long, near the top of each plant, and in addition, the virus was introduced into the stems near the growing points by means of needle punctures. The wiped leaves were numbers 20, 21, and 22, respectively, counting from the bases of the stems.

On Wisconsin-Havana Seed, many chlorotic spots appeared on all wiped leaves and, 6 days after inoculation, mosaic mottling appeared in the new leaves. No signs of local or systemic infection appeared on any of the plants of Ambalema or T.I. 448A during the test. Forty-eight days after inoculation, the T.I. 448A and Ambalema plants were beginning to set seed pods. These plants ranged from 6 to 7½ feet tall, which represented a gain of 3 feet in the length of the main stem after inoculation. At this time, 2 leaves above and 2 leaves below the points of inoculation on 4 plants of T.I. 448A and 3 of Ambalema were assayed. The data are presented in table 5.

These data show that the upper leaves collected from T.I. 448A contained

no measurable virus; the same was true in 2 of the plants of Ambalema. Downward progress in 6 of the 7 plants tested was evident. The virus was not detectable in 4 of the lowermost leaves assayed, and in Ambalema plant No. I there was no evidence of virus in any of the leaves tested. In all virus-containing leaves the virus was in very small amounts.

A second test was conducted with T.I. 448A to determine if the virus might be detected more readily from assays made on the stem cortex. During the winter all leaves on an approximately half-grown T.I. 448A plant, 32 inches tall, were wiped on their tip halves with fresh nondiluted extract of Nicotiana virus 1. Thirty-two days later, the stem was divided into lower and upper groups, the inoculated and the noninoculated portions of the leaves were batched, respectively, and prepared separately, the xylem of the stem was discarded, the cortex only being sampled. All tissues were finely pulped in a mortar and each nondiluted extract was wiped on the primary leaves of 5 bean plants. The data are presented in table 6.

TABLE 6.—*The presence or absence of Nicotiana virus 1 in different tissues of T.I. 448A tobacco when the distal halves of all leaves were inoculated on a plant 30 inches tall. Assays made 32 days after the plants were inoculated. Samples not diluted*

| Sample No. | Location of tissue in the plant | Lesions per bean plant |
|---|---|---|
| | | *Number* |
| 1 | Cortex from top half of stem above inoculated leaves, tip leaves included | 0.0 |
| 2 | Cortex from lower half of stem above inoculated leaves | 0.0 |
| 3 | Cortex from top third of stem in the inoculated zone | 0.2 |
| 4 | Proximal halves of leaves from sample 3 | 0.0 |
| 5 | Inoculated distal halves of leaves from sample 3 | 181.5 |
| 6 | Cortex from central third of stem in the inoculated zone | 0.0 |
| 7 | Proximal halves of leaves from sample 6 | 7.0 |
| 8 | Inoculated distal halves of leaves from sample 6 | 342.0 |
| 9 | Cortex from lower third of stem in the inoculated zone | 0.0 |
| 10 | Cortex from base of stem below inoculated zone | 0.2 |

From these data it appears that the virus progressed very slowly both in leaf and stem of approximately half-grown plants, and it appears that the stem tissues do not support any appreciable increase of the virus. Even though all leaves carried considerable virus in the inoculated tip halves, practically all of it remained in those halves. It is evident that but little increase of virus occurred in tissue outside of the portions inoculated.

A third test was carried out to obtain data on the virus content of roots of middle-age and young plants of T.I. 448A and Wisconsin-Havana Seed tobacco. All plants were inoculated when very young and carried virus in the leaves at the time the roots were assayed. The roots were removed, washed thoroughly, and freed of surface water by means of soft paper. Phosphate buffer solution at pH 7 was added to the roots in double amount by weight and the mass was thoroughly pulped in a mortar. This thick liquid was assayed on 10 bean plants. The number of lesions per bean plant was as follows:

| | | | | |
|---|---|---|---|---|
| T.I. 448A: | 10 | weeks | old | 0.0 lesions |
| do | 15 | do | do | 0.3 do |
| Wisconsin-Havana Seed: | 10 | do | do | 174.7 do |
| do do do | 15 | do | do | 134.3 do |

Like the stem tissues of T.I. 448A tested, the roots of this genotype carried little or no virus. In contrast, the roots of Wisconsin-Havana Seed carried considerable virus.

A fourth test was conducted with T.I. 448A inoculated at six stages of growth, beginning with plants having 7 leaves and ending with plants having about 26 leaves. All plants were from the same seedling (Table 7).

In counting the leaves, those at the base that appeared on the very small seedlings and those at the growing point that were shorter than 2 inches were disregarded. In each inoculation group one leaf near the top on each plant was wiped with nondiluted fresh virus extract from vigorous plants of Wisconsin-Havana Seed tobacco.

The plants were held until maturity. The leaves were assayed at intervals to determine the approximate end point of the virus in the plants. Well developed leaves were used for these exploratory assays because virus is slow to enter the young leaves, and rarely enters the small apical leaves. In accord with Valleau's (9) conclusions, these studies have failed to reveal the presence of virus in detectable quantities in the small central leaves of resistant tobacco plants, except in one instance when a trace was present in a leaf 3 cm. long in a young plant of T.I. 448A which had produced 10 leaves below the tested leaf, and the extracts were diluted to $10^{-4}$ in buffered solution.

Although the small tip leaves of infected Wisconsin-Havana Seed tobacco contain virus, tests have shown that the smallest leaves and primordial tip contain less virus than the leaves which are farther developed. Assays were carried out on leaves from the top of a plant in which mosaic had been fully established for several weeks. Leaves 12, 25, 45, 75, and 125 mm. long induced 55, 234, 276, 217, and 474 lesions per bean plant, respectively. The smallest leaf cited included the tip cluster and the stem primordium. The leaves were consecutive.

From the data in table 7 it will be noted that the end point of the virus is not sharp, especially when the plants were young when inoculated. In proceeding up the plant, the first and second leaves above the inoculated leaf had little or no virus. Each of several (10 to 20) leaves above these contained some virus. Then, in the following 6 to 8 leaves, virus was not regularly present; some leaves had none or had much less than others. Finally, in so far as could be determined by assay, the subsequent leaves were virus-free.

The progress of the virus was appreciable in young plants and it became slight to nil as the plants aged before they were inoculated.

Ordinarily, comparisons cannot be safely made between the results of assays made at different times. However, the conditions of these tests were

such that it is permissible to draw attention to the relatively high virus activity of the wiped leaves of T.I. 448A used in this test, in contrast with the low activity of the wiped leaves of the same tobacco, cited in table 4. The plants cited in table 7 were much younger, even in the last inoculation, than the plants cited in table 4. This seems to be the likely explanation for the differences in activity. In table 3 the relatively high virus activity in the basal-leaf samples from the resistant tobaccos also is explained by the fact that these plants were very young when inoculated, although in this case the basal-leaf samples did not include wiped leaves.

*Virus in Relation to Side-shoots.*—To determine the presence of virus in the side-shoots of plants of T.I. 448A, tests were conducted with moderately young and also with old plants. In one test 5 plants, 12 to 14 inches tall, were cut back to within one inch of the soil line. Bean plants were inocu-

TABLE 7.—*Approximate end points of virus in leaves of T.I. 448A above the inoculated leaf. Leaves numbered from base of stem*

| Position of the inoculated leaf counting from the base of the stem | Position of leaf at end point of virus[a] | Approximate position of leaf at point where virus failed to appear in consecutive leaves | Lesions per bean plant from inoculated tobacco leaves[b] |
|---|---|---|---|
| *No.* | *No.* | *No.* | *No.* |
| 2 | 30 | 22 | 296 |
| 4 | 26 | 18 | 239 |
| 6 | 23 | 15 | ..... |
| 8 | 25 | 17 | 197 |
| 12 | 12 | ..... | 178 |
| 24 | 28 | [c] | 120 |

[a] These data obtained by inoculating bean plants with leaf samples diluted in equivalent amounts of buffered solution.

[b] These extracts were diluted 200 volumes in buffered solution.

[c] There was no evidence of an appreciable increase of virus in the first three leaves above the inoculated leaf. The fourth leaf above induced only 0.3 lesions per bean plant.

lated with a nondiluted extract from the lowest cut-off leaves, and 34 lesions per bean plant were induced. Later the nondiluted extracts from the leaves of the side-shoots of the pruned plants were assayed on bean plants and 33 lesions per bean plant resulted.

In a second test, three, old, infected plants, 48 to 60 inches tall, were cut back, leaving stumps of main stem 12 inches long. One leaf was left at the top of each stump, and 2 side-shoots were allowed to grow from the 2 top buds of each stump. When the top branches were 10 to 12 inches long, the top and bottom leaves of each top shoot and the old leaves subtending them were sampled and assayed on beans. Nondiluted extracts from composited old leaves on the central axis induced 18 lesions per bean plant, but similar extracts from the leaves of the branches induced no lesions.

In a third test a part of the plants were cut off at the soil line and others 12 inches above it. When side-shoots were from 6 to 12 inches long, their

leaves and stem cortices were assayed on bean plants. All samples failed to show virus.

In a fourth test a small side-shoot with 9 leaves was found 2 inches from the soil line on an old, infected plant 50 inches tall. Assays of nondiluted extracts from the leaves of this side-shoot revealed no lesions on bean plants, whereas nondiluted extracts from the 2 lower leaves on the main stem induced 11 and 44 necrotic lesions per bean plant, respectively.

As with the virus-free leaves of the upper main stem, leaves of the side branches of T.I. 448A were found to be capable of increasing the virus. Five leaves of side-shoots on 5 infected plants were sampled by removing a lateral half of each leaf. These samples were assayed on bean plants without dilution, and found to be free from detectable virus. The halves still attached to the side-shoots were then wiped with fresh native virus extract, 11 days later, these half leaves were extracted, and, without dilution, assayed on bean plants. These extracts induced 146, 108, 11, 107, and 60 lesions per bean plant, respectively.

It is obvious from the data presented that virus did not enter side-shoots from the primary stem in measurable amounts except when the shoots developed while the infected plants were young. The leaves of the side-shoots are capable of increasing a limited amount of virus.

*Tests for Starch Patterns.*—Numerous tests were made to determine if the symptomless leaves of T.I. 448A that were known to carry Nicotiana virus 1 might exhibit local disturbances in starch production or starch digestion. Leaves from infected plants, wiped leaves, and virus-free leaves were collected at different times of the day, boiled in water, decolored in boiling alcohol, rinsed in warm water, and stained in the standard starch-testing solutions. In no case was it possible to detect any essential differences between leaves carrying virus and those not carrying it. When the occasional, faint, light-green areas were evident on the infected leaves, the starch-pattern test showed these spots as light areas sometimes containing light-blue centers and faint rings.

*Grafting Test.*—Healthy plants of T.I. 448A and Wisconsin-Havana Seed tobacco were placed with their stems in close proximity in large earthen pots. When the plants were 12 to 14 inches tall, the cortex and cambium were removed from the apposing areas of the stems at a point 6 to 8 inches above the soil line. The cut surfaces were brought in close contact with each other by means of raffia wrappings. The Wisconsin-Havana Seed plants were inoculated with Nicotiana virus 1 and all developed mosaic.

As soon as tissue unions were evident, the stems of T.I. 448A between the soil line and the union were removed. The T.I. 448A plants did not develop mosaic. Leaves from four of the most thrifty cions were assayed after dilution in 3 to 5 parts of buffered solution. The results from one typical cion showed no virus in leaves 30 and 31, traces of virus in leaves 37, 38, 42, 43 and 45, and no virus in the leaves beyond. The traces of virus induced from 0.66 to 20 necrotic lesions per bean plant. Leaves 44, 45 and

46 on the infected stock of Wisconsin-Havana Seed contained sufficient virus to induce 55.6 necrotic lesions per bean plant when diluted to $10^{-5}$ in buffered solution.

In comparison with tables 5 and 7, it will be observed that detectable virus was recovered higher in the cions of T.I. 448A than was the case when the direct inoculation method was used on independent plants.

## DISCUSSION AND CONCLUSIONS

There seems to be little doubt that T.I. 448A is more resistant to the virus of common mosaic (Nicotiana Virus 1) than is the strain of Ambalema tobacco under study. It appears that T.I. 448A greatly reduces the virus reservoir. Failure to find appreciable amounts of virus in the roots and upper leaves, and the relatively low virus content of the lower leaves should greatly reduce the amount of virus overseasoning in these plant residues in the soil.

Chemical analyses carried out by Martin (7) and by Hills (2) indicate that the protein content in the leaves of T.I. 448A infected with Nicotiana Virus 1 is relatively low or even less than it is in leaves of virus-free plants. When the soil was maintained at a high nitrate level, the protein content of the infected leaves was slightly higher than normal, but when the nitrate level was low, the protein content of the infected plants tended to be equal to or below normal. High protein content is undesirable in commercial tobacco, and, from the data available, it appears that this factor will be of little or no consequence in infected commercial tobaccos carrying the resistance of T.I. 448A, especially in the major tobacco areas in the Southern United States, where low levels of nitrogen in the soils are maintained. It is possible that factors for the T.I. 448A type of resistance are present in the Ambalema collections.

Aside from the practical aspects of this survey, the results seem to justify the following conclusions: (1) Nicotiana Virus 1 does not move about freely in resistant plants; (2) virus rarely, if ever, enters the very young leaves of the growing tips of the resistant plants in detectable quantities, especially when the plants have advanced beyond the ten-leaf stage; (3) in T.I. 448A this virus-free zone involves from a fourth to a half of the plant at the time of maturity; (4) virus synthesis in the inoculated leaves of resistant plants tended to be less in the upper leaves of old plants than in the upper leaves of young plants, and the upper leaves of old plants tended to be more resistant than the lower active leaves, indicating that the successive leaves are increasingly resistant to virus synthesis. The basis for this resistance is not known. When leaves are inoculated by wiping, the number of vulnerable points for entry and the susceptibility of these points must be taken into consideration.

The evidence obtained in the grafting test indicates that the virus did move farther in T.I. 448A cions on infected Wisconsin-Havana Seed plants, than was the case when young T.I. 448A plants were inoculated directly.

When one considers the large quantities of virus in the susceptible Wisconsin-Havana Seed plants, this observation is not surprising. However, it is clearly evident from the data that little virus reached the upper leaves of the resistant cions.

This observation and the results from the direct inoculations suggest that the channels of virus flow are restricted in some manner in T.I. 448A. On the other hand, it is quite possible that these channels in resistant plants are in no way different from those in susceptible plants. If this is true, it would appear that the distance which the majority of virus particles travel in the plant tends to be limited to a considerable degree, and that movement over long distances is accomplished, for the most part, by stages between zones of synthesis and by relatively few particles that get into the main vascular channels. After the virus enters the growing-point zone of the stem in highly susceptible plants, the problem of movement into all subsequent leaves is greatly reduced, in comparison with the situation in resistant plants whose growing points seldom, if ever, receive virus from the zones of synthesis.

Holmes (3), working with common mosaic in Turkish tobacco, a very susceptible type, presented data which make it appear that the bulk of the virus tends to remain in or very near the infection sites for a long time, a small amount trickling out to start new infection sites. If a similar situation obtains in T.I. 448A, with its low level of virus synthesis, it is evident that the amount of virus moving out of the infection sites gradually approaches the zero point as infection proceeds in the increasingly resistant leaves of the growing plant, and in the test cited in table 5, the more rapid progress of the virus down the plant may have been facilitated by the apparently greater susceptibility of the leaves as we proceed in that direction (Table 4).

Resistant tobacco is of value for studies on virus movement, but further study will be required to determine if the results obtained with resistant plants can be applied directly to highly susceptible plants.

To the student of gross pathology, T.I. 448A falls in the class of so-called symptomless carriers, and is immune from the mosaic disease. However, from the standpoint of those who are concerned with the solution of the basic problems encountered in resistance and immunity, the interest must go beyond gross symptoms. These investigators must concern themselves with the minor as well as the major signs of disease in resistant and in highly susceptible genotypes. When the problem is approached in such a manner, it is difficult to escape the conclusion that natural immunity should be determined primarily on the basis of nonsupport of a pathogen rather than on a basis of symptoms. In T.I. 448A, the virus induces slight changes (2) in the oxidase, catalase, peroxidase and protein, but as slight as these changes are, they do represent signs of disease. As indicated in an earlier note (5), it seems unlikely that any so-called symptomless carrier is totally free from signs of disease.

BUREAU OF PLANT INDUSTRY STATION,
BELTSVILLE, MD.

LITERATURE CITED

1. CLAYTON, E. E., H. H. SMITH, and H. H. FOSTER. Mosaic resistance in *Nicotiana tabacum* L. Phytopath. 28: 286–288. 1938.
2. HILLS, CLAUDE H., and H. H. MCKINNEY. The effect of mosaic-virus infection on the protein content of susceptible and resistant strains of tobacco. Phytopath. 32: 857–866. 1942.
3. HOLMES, FRANCIS O. Local and systemic increase of tobacco mosaic virus. Amer. Jour. Bot. 17: 789–805. 1930.
4. MCKINNEY, H. H. Comparative studies on two genotypes of *Nicotiana tabacum* resistant to Nicotiana Virus 1. Phytopath. (Abstract) 29: 16. 1939.
5. ————. Virus antagonism, natural host resistance, and the acquired-immunity concept. Phytopath. 31: 1059–1061. 1941.
6. ————, and L. W. BOYLE. Culture and inoculation procedure for *Phaseolus vulgaris* L., when used to test the activity of tobacco common-mosaic virus. (In preparation.)
7. MARTIN, LAWRENCE F., A. K. BALLS, and H. H. MCKINNEY. The protein content of mosaic tobacco. Science 87: 329–330. 1938.
8. NOLLA, J. A. B., and A. ROQUE. A variety of tobacco resistant to ordinary tobacco mosaic. Jour. Dept. Agr. Puerto Rico Univ. 17: 301–303. 1933.
9. VALLEAU, W. D. Localization and resistance to tobacco mosaic, in *Nicotiana*. Ky. Agr. Exp. Stat. Bull. 360. pp. 202–230. 1935.

# GROWTH OF PHYTOPATHOGENIC BACTERIA IN A SYNTHETIC ASPARAGIN MEDIUM

MORTIMER P. STARR AND JAMES E. WEISS
With the technical assistance of
HAROLD P. KLEIN AND CHARLOTTE B. SISSELMAN

(Accepted for publication August 8, 1942)

During an investigation of the nutrition of phytopathogenic bacteria, it became necessary to use a variety of synthetic media. In this connection, experiments were performed to determine for the growth of the bacterial phytopathogens the suitability of a medium containing asparagin as the sole source of both carbon and nitrogen.

Use of synthetic asparagin media is not new to bacterial plant pathology. Smith (14, 15) records several cases where asparagin sufficed as the nitrogen source for phytopathogenic bacteria. Clara (4) reports that the 19 species of green-fluorescent pigment-producing plant pathogenic bacteria that he tested, grew in a medium containing asparagin as the only carbon and nitrogen source. Burkholder (2) states that the members of the yellow-pigmented "*Phytomonas campestris* group" do ". . . not utilize asparagin as a source of carbon and nitrogen, as the green fluorescent [phytopathogenic *Pseudomonas*] forms do . . . " and, in a personal communication, explains that this statement is based on unpublished observations he had made of the growth of these species in Clara's (4) asparagin medium. As has been noted (1, 2, 3, 6, 7, 16), the genus *Phytomonas* Bergey *et al.* is composed of several unlike groups of phytopathogenic bacteria. Hence, it appeared likely that confirmation of the foregoing and an extension that would include representatives of all the different groups comprising that outmoded genus, would aid in establishing the validity of the groupings that have been recommended (2, 6, 7, 16).

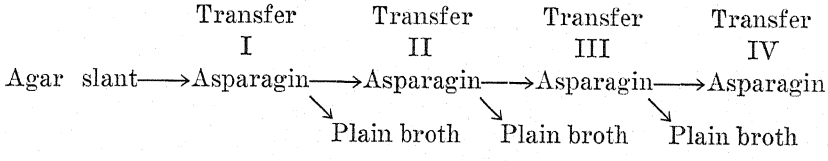
## EXPERIMENTAL

One hundred and seventy-three isolates of plant-pathogenic bacteria, consisting of 66 species and varieties, were tested for their ability to grow when transferred repeatedly in a synthetic asparagin medium. In the main, these isolates are the same as those cultured by Starr and Burkholder (16) in their studies on lipase activity; reference to that publication will give additional information concerning each culture.

The medium used in these experiments consisted of 0.1 per cent $KH_2PO_4$, 0.02 per cent KCl, 0.02 per cent $MgSO_4 \cdot 7H_2O$, 0.5 per cent asparagin, pH 6.4. In preparing this medium, a suitably concentrated solution of the inorganic salts was tubed and sterilized by autoclaving. To this was added aseptically the proper amount of a concentrated solution of asparagin, sterilized by filtration through an L5 Chamberland candle. The resulting medium was incubated before use to assure sterility.

Throughout this investigation, all glassware was cleaned by soaking in dichromate-sulphuric acid cleaning solution for at least 24 hours, washing thoroughly in tap water, and then rinsing several times in distilled water. Careful cleaning is essential in order to eliminate the minute quantities of organic matter that ordinary washing does not remove and which might be used by the bacteria in lieu of asparagin.

Actively growing cells from agar slant cultures were transferred to the synthetic asparagin medium. To avoid contamination of the synthetic medium with unknown substances that might support growth instead of the asparagin, the inocula were kept small and care was taken to carry over cells and not agar. It is inevitable, however, that certain metabolites associated with the cells will be introduced; these might account for growth in the first transfer. Since it is the opinion of experts in the field of bacterial nutrition (9, 10) that this added material can be eliminated by several successive transfers in the medium, the asparagin cultures were transferred serially by wire loop to fresh asparagin at 4- to 6-day intervals. To prove that failure to grow in the asparagin medium is to be attributed to the insufficiency of this medium rather than to dead inocula, transfers usually were made at the same time to plain nutrient broth as follows:




In all cases, the organisms grew in these nutrient broth controls.

All cultures were incubated at 27° C. and examined daily for growth. Growth was defined as a distinct turbidity within 6 days after inoculation and was checked by gram staining the last culture in each series. The results of these experiments are presented below.

For reasons discussed in detail elsewhere (2, 16), the generic names of the species listed in the following paragraphs have been changed from *Phytomonas* to those now considered more appropriate; *viz.*, *Xanthomonas* Dowson (6) for the members of the yellow-pigmented "*Phytomonas campestris* group" (2); *Pseudomonas* Migula for the green-fluorescent pigment-producing and related phytopathogenic bacteria (4, 6, 12); *Agrobacterium* Conn (5) for the members of the tumor-forming "*Phytomonas tumefaciens* group"; *Corynebacterium* Lehmann and Neumann for certain of the gram-positive forms (7, 8, 13, 17); *Phytomonas* Bergey *et al.* is retained for those phytopathogenic bacteria still of doubtful systematic position.

The notation after each name is the designation now used for the isolate in the stock-culture collections of W. H. Burkholder, Cornell University, and of the Bacteriological Laboratory at Brooklyn College. The number in parenthesis refers to the designation used for that culture by Starr and Burkholder (16).

The phytopathogenic bacteria that grew through 4 successive transfers in the synthetic asparagin medium are: *Agrobacterium gypsophilae* TG1 (NB), *A. radiobacter* TR1 (H), *A. savastanoi* TS3 (COS), *A. savastanoi* var. *fraxini* TS1 (NB), *A. tonellianum* TT1 (COS), *A. tumefaciens* TT2 (B2); TT3 (B6), *Corynebacterium fascians* CF1 (R); CF2 (S), *Phytomonas stewartii* SS2 (S16); SS3 (S15); SS6 (B93); SS8 (B95); SS9 (B96); SS11 (3152); SS12 (3153); SS13 (3154), *Pseudomonas alliicola* PA5 (4); PA6 (1), *P. angulata* PA3 (B99); PA11; PA12, *P. atrofaciens* PA13; PA14, *P. berberidis* PB3, *P. caryophylli* PC3 (3); PC8 (R8), *P. cichori* PC1 (G18), *P. coronafaciens* PC17; PC18, *P. delphinii* PD1 (G36), *P. glycinea* PG1; PG2; PG3; PG4; PG5; PG6; PG7, *P. jaggeri* PJ1, *P. lachrymans* PL1 (G12); PL3, *P. lapsa* PL2 (Ark), *P. maculicola* PM1 (G37), *P. medicaginis* var. *phaseolicola* PM3 (G3); PM4 (G31); PM5 (G29); PM7, *P. mellea* PM8, *P. papulans* PP4, *P. pisi* PP1 (G32), *P. polycolor* PP2 (G11), *P. primulae* PP3 (Ark), *P. solanacearum* PS14; PS15, *P. striafaciens* PS1 (70), *P. syringae* PS2 (B38); PS3 (B45); S4 (B70); PS5 (SyF); PS6 (G21); PS7 (G28); PS8 (G24); PS9 (Syl); PS11 (G20); PS12 (G19); PS13 (G7), *P. tabaci* PT1; PT3; PT4; PT5; PT6, *P. tomato* PT7; PT8, *P. viburni* PV1 (1); PV3 (3), *P. viridiflava* PV5 (G5), *P. viridilivida* PV4 (G23).

The phytopathogenic bacteria that did not grow in the synthetic asparagin medium are: *Agrobacterium rubi* TR2; TR3, *Corynebacterium flaccumfaciens* CF3[a] (S1); CF6 (S4); CF8[a] (S6); CF9 (S7); CF10 (S9); CF11 (S20); CF12 (S31), *C. insidiosum* C11 (S23); C12 (S21); C13 (S34), *C. michiganense* CM1 (S29); CM2 (S18); CM3 (S30); CM4 (S11), *C. poinsettiae* CP1; CP2; CP3; CP5; CP13; CP14; CP20; CP21; CP27; CP31; CP35; CP37; CP39; CP41, *Phytomonas manihotis* SM1 (R2); SM2 (R3); SM3 (R4); SM5 (4); SM6 (R1); SM9, *P. mors-prunorum* PM6 (B69), *P. stewartii* SS1[a] (S13); eight isolates *do* grow (*cf.* preceding list and text), *P. tardicrescens* ST1 (S26), *Xanthomonas barbareae* XB1 (B1); XB2 (BR), *X. begoniae* XB3 (96); XB6, *X. campestris* XC1 (85); XC2 (86); XC3 (R4), *X. campestris* var. *armoraciae* XC4 (HI), *X. corylina* XC5[a] (78), *X. geranii* XG1 (2); XG2 (3); XG3 (5); XG4 (G1); XG5 (87); XG6 (89), *X. gummisudans* XG7 (94), *X. hederae* XH1 (16), *X. incanae* XI1 (St1); XI2 (St2); XI3 (St3), *X. juglandis* XJ1 (77); XJ2 (79), *X. malvacearum* XM1[a] (1); XM2[a] (B87), *X. papavericola* XP5 (47), *X. pelargonii* XP6 (92); XP7[a] (93); XP8[a] (100), *X. phaseoli* XP1 (H); XP2 (B16); XP4 (E2); XP14 (MPS), *X. phaseoli* var. *sojense* XP3 (11), *X. pruni* XP9[a] (B65); XP10[a] (1); XP11[a] (2); XP12[a] (3); XP13[a] (B67), *X. translucens* XT1 (90), *X. translucens* f. sp. *hordei-avenae* XT6, *X. translucens* var. *undulosa* XT4 (91); XT5 (83), *X. vasculorum* XV1[a] (49), *X. vesicatoria* XV3 (1); XV5 (3); XV6 (4); XV7 (5); XV8 (6); XV9 (7); XV10 (8); XV11[a] (13); XV12 (38); XV13 (L6); XV14 (99); XV15[a] (VI), *X. vesicatoria* var. *raphani* XV16 (L11), *X. vitians* XV2 (14).

[a] This isolate sometimes forms a scanty turbidity in the first transfer to the synthetic asparagin medium, but does not grow at all on subsequent transfers in this medium. Moreover, the quantity of growth, in these cases, is less than that produced by the organisms listed in the preceding paragraph.

## DISCUSSION

The data presented in the foregoing show that the members of the outmoded genus *Phytomonas* Bergey *et al.* vary in ability to grow in a medium containing asparagin as their only source of both carbon and nitrogen. It should, however, be recognized that certain of the groups comprising this genus act characteristically in this medium: all 60 isolates of phytopathogenic *Pseudomonas* grow well on repeated transfer in the synthetic medium, while none of the 57 *Xanthomonas* isolates can do so. This confirms the work of Clara (4) and of Burkholder (2) mentioned above. It must be noted, however, that some of the *Xanthomonas* isolates do product a slight turbidity in the first transfer in the synthetic asparagin medium. This growth probably occurs at the expense of nutrients carried over with the inocula, which are frequently heavy in this group because of the slimy nature of the growth on agar. There is no growth of any of the *Xanthomonas* species on subsequent transfer in the asparagin medium and, moreover, the scanty turbidity produced by these species in the first transfer at no time approaches in quantity that made by the *Pseudomonas* isolates.

The genus *Agrobacterium* was recently established in the Rhizobiaceae by Conn (5) to include the gall-forming phytopathogenic bacteria and related soil forms. Isolates of the type species, *Agrobacterium tumefaciens,* as well as of *A. radiobacter, A. tonellianum, A. gypsophilae,* and *A. savastanoi* grow well in the synthetic asparagin medium. Accordingly, it is interesting that the cane gall organism, *A. rubi,* which is otherwise closely related to *A. tumefaciens,* cannot grow at all in the asparagin medium. Relevant to this is Pinckard's (11) observation that *A. rubi* grew only slightly in a glucose-asparagin medium in which other members of this group grew well.

When one considers the relatively complex nutritive requirements of *Corynebacterium diphtheriae* (10), it is not surprising that the phytopathogenic *Corynebacterium* species fail to grow with asparagin as the only source of carbon and nitrogen. The only exception is *C. fascians,* which grows rapidly and luxuriantly in this medium; perhaps this species is not now classified correctly.

Dowson (6) placed the corn-wilt bacterium, *Phytomonas stewartii,* in the genus *Xanthomonas,* although Burkholder had excluded it from the equivalent "*Phytomonas* proper" group (3). This organism possesses certain characteristics which differentiate it from most species of *Xanthomonas, viz.,* lack of flagella (3, 15), failure to peptonize milk (3, 15) or to produce hydrogen sulphide, inability to attack cottonseed oil as shown by the spirit blue agar technique (16). To these differences, there now can be added the ability of *P. stewartii* to grow in a medium in which asparagin is the sole carbon and nitrogen source, as contrasted with the inability of typical *Xanthomonas* species to grow under identical conditions. This asparagin medium is not completely suitable for the maximal development of *P. stewartii* since growth was rather slow and, indeed, one atypical isolate (SS1) did not grow after the first transfer. However, some significance

must be attached to the fact that 8 of the 9 *P. stewartii* cultures tested produced a distinct growth through 4 successive transfers in the synthetic asparagin medium while no typical *Xanthomonas* isolate ever did. For the aforementioned reasons, it is recommended that the corn-wilt bacterium be excluded from *Xanthomonas* and that the name *Phytomonas stewartii* be retained until such time as the exact taxonomic position of that species be determined.

SUMMARY

A series of 173 isolates of phytopathogenic bacteria, consisting of 66 different species and varieties, was tested for the ability to grow through 4 successive transfers in a synthetic medium that contained asparagin as the only source of both carbon and nitrogen. Certain of the genera and groups comprising the outmoded genus *Phytomonas* Bergey *et al.* have a distinctive action on this medium. Accordingly, this characteristic may be valuable in classifying the bacterial plant pathogens.

BACTERIOLOGICAL LABORATORY,
BROOKLYN COLLEGE,
BROOKLYN, NEW YORK

LITERATURE CITED

1. BURKHOLDER, W. H. The genus *Phytomonas*. Phytopath. 20: 1–23. 1930.
2. ———. The taxonomy and nomenclature of the phytopathogenic bacteria. Phytopath. 29: 128–136. 1939.
3. ———. *Phytomonas* Bergey *et al.* *IN* Bergey's Manual of Determinative Bacteriology. Fifth edition, pp. 142–218. Baltimore, 1939.
4. CLARA, F. M. A comparative study of the green-fluorescent bacterial plant pathogens. N. Y. (Cornell) Agr. Exp. Stat. Mem. 159: 1–36. 1934.
5. CONN, H. J. Validity of the genus *Alcaligenes*. Jour. Bact. 44: 353–360. 1942.
6. DOWSON, W. J. On the systematic position and the generic names of the Gram-negative bacterial plant pathogens. Zentralbl. Bakt. u.s.w. Abt. II 100: 177–193. 1939.
7. ———. On the generic name of the gram-positive bacterial plant pathogens. British Mycol. Soc. Trans. 25: 311–314. 1942.
8. JENSEN, H. L. Studies on saprophytic mycobacteria and corynebacteria. Linn. Soc. N.S.W. Proc. 59: 19–61. 1934.
9. KOSER, S. A., and F. SAUNDERS. Accessory growth factors for bacteria and related microorganisms. Bact. Rev. 2: 99–160. 1938.
10. MUELLER, J. H. Nutrition of the diphtheria bacillus. Bact. Rev. 4: 97–134. 1940.
11. PINCKARD, J. A. Physiological studies of several pathogenic bacteria that induce cell stimulation in plants. Jour. Agr. Res. [U.S.] 50: 933–952. 1935.
12. SELEEN, W. A. A study of green-fluorescent pigment-producing organisms. Thesis, Cornell University. 1941.
13. SKAPTASON, J. B., and W. H. BURKHOLDER. Classification and nomenclature of the pathogen causing bacterial ring rot of potatoes. Phytopath. 32: 439–441. 1942.
14. SMITH, E. F. Bacteria in relation to plant diseases. Vols. 1–3. Washington, 1905, 1911, 1914.
15. ———. An introduction to bacterial diseases of plants. Philadelphia, 1920.
16. STARR, M. P., and W. H. BURKHOLDER. Lipolytic activity of phytopathogenic bacteria, determined by means of spirit blue agar, and its taxonomic significance. Phytopath. 32: 598–604. 1942.
17. ———, and P. P. PIRONE. *Phytomonas poinsettiae* n.sp., the cause of a bacterial disease of poinsettia. Phytopath. 32: 1076–1081. 1942.

# A BOTRYTIS DISEASE OF LUPINES[1]

J. L. WEIMER

(Accepted for publication July 28, 1942)

## INTRODUCTION

For the past few years 3 species of imported lupines (*Lupinus angustifolius, L. albus* and *L. luteus*) have been grown experimentally in the Southeastern United States, and *L. angustifolius* has become fairly well established as a winter cover crop. Owing to their lack of winter hardiness lupines can be grown only over a very limited area and, even then, they may be severely injured in cold winters. In February, 1938, following a January freeze, which injured some lupine plantings severely, a disease was quite abundant at Tifton, Georgia, and to a less extent at Gainesville, Florida. Whether the disease was actually responsible for the dying of the plants or was simply following the freezing injury was not clear. The nature and severity of the disease, however, seemed to justify investigation, and it is the purpose of this paper to record the consequent results.

## SYMPTOMATOLOGY

Large plants, some already in bloom, showed all degrees of injury. Some had been dead for a considerable period, others were dwarfed, and still others had made good growth and then were being partly or entirely killed. An examination showed that cankers, sometimes several inches long, in the main stem near the soil or at different distances above, were girdling the stem and causing death of the part above. In some instances only a portion of the top or small branches were dying. The cankers usually, if not always, had a small infected or dead stem at or near the center, suggesting that this was the point of attack that resulted in the death of the part affected. Sometimes, leaflets, petioles, or stipules, were involved. Stem girdling eventually resulted in the wilting and death of the parts above. When a leaflet or other small organ was attacked, it died rapidly, and the petiole or branch and often the large branches or the main stem itself became involved and killed. Something of the nature of the injury is apparent in figure 1, A. Here are shown 2 large plants, the one at the right is healthy and that on the left is wilting and the leaves drooping because of a large canker near the ground, not evident in the picture. The cankers are usually greenish to brown and may be smooth or have a grayish coating of fungous mycelium and conidiophores. The nature of the cankers is shown in figure 1, C, and an enlargement of the same cankers after being held in the moist chamber for 4 days is shown in figure 1, D. The stem at the left in figure 1, C, shows small dead branches that served as the avenue through which

[1] Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Georgia Agricultural Experiment Station, Experiment, Georgia. Paper No. 100, Journal Series, Georgia Agricultural Experiment Station.

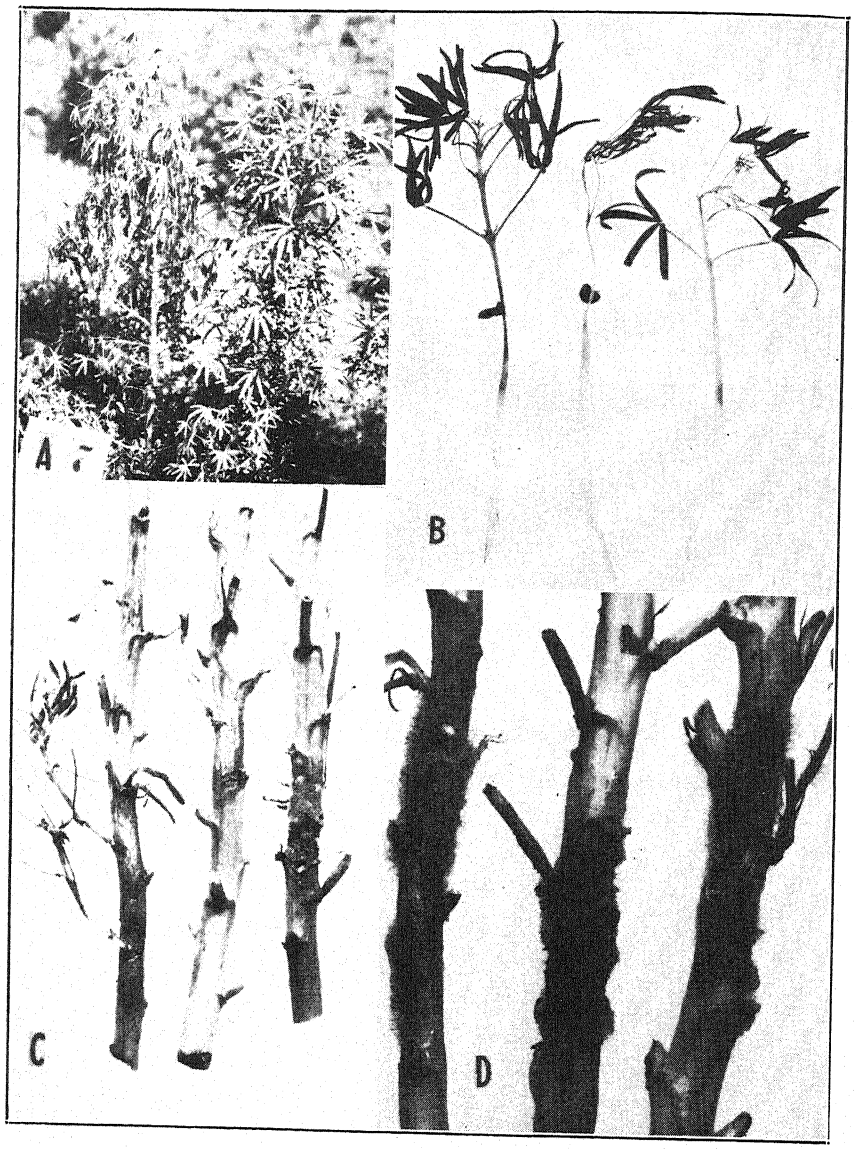



FIG. 1. *Botrytis cinerea* on *Lupinus angustifolius*. A. Two large lupine plants growing in a field at Gainesville, Fla. The plant at the left shows wilting of the leaves and bending over of the terminal blossom cluster due largely to girdling of the stem. In a few instances separate infections had occurred in the young leaflets at the ends of branches. The plant at the right is healthy. $\times \frac{1}{12}$. B. The 2 young lupine plants at the left have been infected through the cotyledons. The center plant also had some infected leaflets. The plant at the right was infected through very young leaflets near the terminal bud. $\times \frac{1}{8}$. C. The canker at the left was caused by infection of the stem through the side branches. The stem at the right shows some compact fungus growth, such as is sometimes seen during medium dry periods. Often no fungus is apparent on the surface of the canker. $\times \frac{1}{2}$. D. Such cankers as in C covered with the aerial mycelium and conidiophores of the *Botrytis* after 3 or 4 days of wet weather. $\times$ 12/13.

the larger stem was invaded. Similar dead twigs are almost always present in the cankers. The stem at the right shows the fungus fruiting on the surface as it does under moist conditions, such as during a rainy period. A clearer view of what these cankers look like during a wet spell is shown in figure 1, D. Here the fungus has come out of the tissue and fruited over the surface of the lesion.

## ETIOLOGY

A study of the fungus fruiting on the surface of the lesions and isolated from the inner tissues showed that it was a species of *Botrytis*. Inoculations were made to determine whether this fungus was able to attack healthy plants or simply followed into frozen tissue. Field evidence seemed to support both possibilities. The disease is seen in the field, at least, more commonly following a cold winter. Nevertheless there seemed to be instances where it was attacking young twigs that had developed since any frost had occurred.

Inoculation experiments were made in which a heavy suspension of spores from a pure culture of the fungus in tap water was atomized onto 10 seedlings of *Lupinus angustifolius* that varied in size from just out of the soil to 3 inches tall. The terminal buds of some plants were not yet exposed, while the first true leaves of other plants were fairly well-developed. The plants were held at laboratory temperature (60–75° C.) under bell jars for 72 hours. No infection resulted in any of the plants. The cause for the total lack of infection was not evident but it was thought that the mean temperature might have been too low.

Since *Botrytis* belongs to a group of fungi some of which need a saprophytic start before being able to invade healthy tissue, the above experiment was repeated but with bits of inoculum (hyphae) with or without agar attached. The mycelial felt was broken up into small pieces in water and applied to the plants with a brush. Four days after these inoculations were made 3 plants out of 9 showed infection. The fungus grew from the pieces of inoculum into the tissue and caused a rapid decay of the invaded parts. In some cases petioles were entirely decayed through and the leaves wilted; in other instances the fungus had entered the plant through the leaf axil; in still others the cotyledons were invaded. In one pot 5 out of 10 plants were infected. Infection took place through the leaf blade, petiole, stipule, terminal bud, or cotyledons, but not through the main stem. The latter was invaded and killed, but only after the fungus had first attacked some young tender part serving as a point of entry. Figure 1, B, shows 3 of the inoculated plants. The 2 at the left have been invaded through the cotyledons, while the terminal bud of the plant at the right was killed. The fungus was reisolated from the decayed tissue and its parasitism again proved. None of the control plants were attacked.

These experiments show that *Botrytis* can attack young healthy plants and produce symptoms similar to those seen in the field. No freezing or

other injury is necessary. It is believed, however, that a high humidity is essential and it also seems probable that infection can take place, more readily at least, if some provision is at hand to permit the fungus to get a saprophytic start. For example, pieces of infected tissue broken from a diseased plant might become lodged in the axil of a leaf and under conditions of high humidity the fungus grow from the diseased into the healthy tissue. It is possible that spores might fall onto a part of a healthy plant where dirt or organic matter has accumulated that would provide the necessary food for the beginning of active growth. The fungus also may find suitable conditions for infection in the organic matter at or near the surface of the soil. Frozen tissue, under conditions suitable for the rapid development of the fungus, doubtless provides an ideal infection court. This accounts for the abundance of the disease following a freeze when the fungus can attack many parts of the plant and greatly augment the damage done by low temperature.

The causal fungus is easily isolated and grows well on all media tried by the writer. On potato agar with 2 per cent dextrose added, there develops a rather dense grayish mat, nearly black next to the medium in the lower half of the slant. Little or no fruiting occurs on this medium. On oatmeal agar the fungus forms a loose hyphal growth instead of the dense mat, as on potato dextrose agar. A large part or all of the space between the agar slant and the wall of the tube usually is filled with the loose grayish mycelium, similar to that seen on the cankers during wet weather. The fungus, ordinarily, does not sporulate abundantly on culture media. It fruits fairly well, however, when grown on oatmeal agar on a long slant in a large test tube. The spores range from 8.4–13.65 × 6.8–10.5 μ (average of 50 spores 10.6 × 8.2 μ). The mycelium on oatmeal agar ranges from 8 to 12.6 μ in diameter.

A study of the fungus leaves little doubt that it belongs in the *Botrytis cinerea* group.[2] It has the typical cinerea type of conidiophore. Irregular, mid-size, black sclerotia are produced rather sparingly in culture and probably in or on the host, although they were not seen on the latter. These sclerotia, no doubt, serve to carry the fungus over from one crop season until the next. No apothecial stage has been seen.

What appears to be the same disease is described by Curtis[3] in New Zealand, by Dippenaar[4] in South Africa, and by Pape[5] in Germany. The disease probably is coextensive with the crop.

## CONTROL

As is the case of diseases caused by other soil-borne fungi, the botrytis

[2] This conclusion was confirmed by H. H. Whetzel, Cornell University, Ithaca, N. Y., who kindly examined cultures of the fungus.

[3] Curtis, K. M. Two fungal diseases of blue lupine. New Zealand Jour. Agr. 26: 240–246. 1923.

[4] Dippenaar, B. J. Drie siektes wat in Suid-Afrika op Lupienplante voorkom. Ann. Univ. Stellenbosch Ser. B. 9, 1: 3–10. 1931.

[5] Pape, H. Krankheiten and Schädlinge der Lupine. Landw. Zeit. 47: 316–318. 1927.

disease of lupines is difficult to control. Certain rather obvious precautions may aid in reducing losses. Some of these are: practice rotation, avoid wet areas, do not plant seed too thick, use only the hardiest varieties available, and do not attempt to grow the crop too far north until more winter-hardy selections are available.

## SUMMARY

A disease of lupines caused by a fungus of the *Botrytis cinerea* group is described. This disease characteristically produces cankers on the stems or branches, often girdling them and killing the parts above. Although not essential for infection, frozen tissue forms an excellent infection court and damage caused by late spring freezes may be considerably augmented by the fungus.

# PHYSIOLOGIC RACES OF USTILAGO NIGRA[1]

V. F. TAPKE

(Accepted for publication June 16, 1942)

*Ustilago nigra* Tapke, described in 1932 as a seedling-infecting loose smut of barley (2), is now apparently as widespread and damaging in the United States as *U. nuda* (7), the well-known floral-infecting loose smut of barley. The estimated annual loss caused by these smuts (7) amounts to 2,000,000 bushels, a loss that now may be halved through seed treatment with certain easily applied surface disinfectants, as *U. nigra* is readily controlled by these chemical treatments (2, 3, 7). The problem of control, however, may be greatly simplified through the use of varieties resistant to both *U. nuda* and *U. nigra*. To aid in breeding for resistance against *U. nigra*, a study of physiologic races in this species, therefore, was begun. The present report embraces the results of the 7-year period, 1935–1941.

## EARLIER RESEARCH

Two physiologic races in *Ustilago nigra* were first reported in 1936 (4). In a recent abstract, Josephson (1) notes the occurrence of 5 distinct races and possibly 3 others in a barley loose smut referred to as *U. medians* (*U. nigra*) (6, 7).

## MATERIALS AND METHODS

The experiments reported herein were conducted at Ithaca, New York, in 1935 and from 1937 to 1941, inclusive.[2] The technique was the same as that described in a study of physiologic races of covered smut of barley (*Ustilago hordei*) (5). The differential hosts were pure lines of Excelsior (C.I. 1248), Hannchen (C.I. 531), Himalaya (C.I. 1312), Lion (C.I. 923), Nepal (C.I. 595), and Odessa (C.I. 934). One hundred and sixty-eight collections of *U. nigra* were tested, including the 10 studied in 1935 on which a previous report was made (4). The percentages of smut were based on counts of 300 heads per row in duplicate seedings each year.

As the different races were isolated, a type collection of each was selected and continued in the later tests. The inoculum of each race was collected from the variety that most clearly differentiated it from others. The different races were identified by the general resistance or susceptibility of the differential varieties.

## EXPERIMENTAL RESULTS

Seven distinct physiologic races were isolated in the study of the 168 collections of *Ustilago nigra*. The reaction of a type collection of each race,

[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the New York (Cornell) Agricultural Experiment Station.

[2] For supervision of the field plantings at Ithaca, and for other helpful assistance, the writer is indebted to Dr. H. H. Love and Mr. W. T. Craig, Department of Plant Breeding at Cornell University.

beginning with the year in which it was isolated, is presented in table 1. Races 4 and 5 are the two described in 1936, but not numbered (4). The results show consistent differences in the pathogenicity of the different races. It is evident that conditions for smut were better in some years than in

TABLE 1.—*Percentages of loose smut produced on six differential varieties by seven physiologic races of Ustilago nigra*

| Race No. | Year | Smutted heads in | | | | | | Race identified mainly by the susceptibility of |
|---|---|---|---|---|---|---|---|---|
| | | Excelsior C.I. 1248 | Hannchen C.I. 531 | Himalaya C.I. 1312 | Lion C.I. 923 | Nepal C.I. 595 | Odessa C.I. 934 | |
| | | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | |
| 1 | 1937 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 4.0 | Odessa |
| | 1938 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 36.0 | |
| | 1939 | 0.0 | 0.0 | 0.0 | 0.0 | 5.0 | 65.0 | |
| | 1940 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 42.0 | |
| | 1941 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 29.0 | |
| 2 | 1937 | 0.0 | 12.0 | 0.0 | 0.0 | 0.0 | 24.0 | Hannchen and Odessa |
| | 1938 | 0.0 | 42.0 | 0.0 | 0.0 | 0.0 | 53.0 | |
| | 1939 | 0.0 | 51.0 | 0.0 | 0.0 | 0.0 | 65.0 | |
| | 1940 | 0.0 | 24.0 | 0.0 | 0.0 | 0.0 | 49.0 | |
| | 1941 | 0.0 | 15.0 | 0.0 | 0.0 | 0.0 | 17.0 | |
| 3 | 1938 | 0.0 | 0.0 | 0.0 | 20.0 | 0.0 | 55.0 | Lion and Odessa |
| | 1939 | 0.0 | 1.0 | 0.0 | 17.0 | 0.0 | 81.0 | |
| | 1940 | 0.0 | 0.0 | 0.0 | 9.0 | 0.0 | 57.0 | |
| | 1941 | 0.0 | 0.0 | 0.0 | 4.0 | 0.0 | 33.0 | |
| 4 | 1935 | 0.0 | 33.0 | 0.0 | 37.0 | 0.0 | 60.0 | Hannchen, Lion and Odessa |
| | 1937 | 0.0 | 20.0 | 0.0 | 16.0 | 0.0 | 55.0 | |
| | 1938 | 0.0 | 29.0 | 0.0 | 19.0 | 0.0 | 62.0 | |
| | 1939 | 0.0 | 46.0 | 1.0 | 24.0 | 2.0 | 82.0 | |
| | 1940 | 0.0 | 33.0 | 0.0 | 22.0 | 0.0 | 55.0 | |
| | 1941 | 0.0 | 30.0 | 0.0 | 17.0 | 0.0 | 35.0 | |
| 5 | 1935 | 0.0 | 16.0 | 17.0 | 3.0 | 32.0 | 48.0 | Hannchen, Himalaya, Lion, Nepal and Odessa |
| | 1937 | 0.0 | 29.0 | 28.0 | 8.0 | 43.0 | 39.0 | |
| | 1938 | 0.0 | 9.0 | 19.0 | 13.0 | 20.0 | 45.0 | |
| | 1939 | 0.0 | 28.0 | 36.0 | 15.0 | 54.0 | 70.0 | |
| | 1940 | 0.0 | 25.0 | 28.0 | 11.0 | 21.0 | 52.0 | |
| | 1941 | 0.0 | 8.0 | 10.0 | 3.0 | 5.0 | 18.0 | |
| 6 | 1937 | 21.0 | 8.0 | 13.0 | 9.0 | 35.0 | 28.0 | All six varieties |
| | 1938 | 16.0 | 7.0 | 16.0 | 16.0 | 14.0 | 30.0 | |
| | 1939 | 44.0 | 19.0 | 22.0 | 7.0 | 53.0 | 58.0 | |
| | 1940 | 30.0 | 12.0 | 19.0 | 11.0 | 22.0 | 44.0 | |
| | 1941 | 7.0 | 3.0 | 6.0 | 3.0 | 6.0 | 18.0 | |
| 7 | 1937 | 0.0 | 26.0 | 23.0 | 0.0 | 27.0 | 40.0 | Hannchen, Himalaya, Nepal and Odessa |
| | 1938 | 0.0 | 13.0 | 12.0 | 0.0 | 22.0 | 30.0 | |
| | 1939 | 0.0 | 47.0 | 16.0 | 0.0 | 29.0 | 65.0 | |
| | 1940 | 0.0 | 9.0 | 12.0 | 0.0 | 28.0 | 35.0 | |
| | 1941 | 0.0 | 11.0 | 20.0 | 0.0 | 26.0 | 24.0 | |

others. In 1939, for example, infection percentages ran much higher than in 1941. Despite this variability, however, the identity of each of the 7 races was clearly maintained. Under the favorable conditions of 1939 some varieties, such as Nepal, were, that year only, lightly smutted by certain races.

The frequency of occurrence and the distribution by States of the 7 races of *Ustilago nigra* are presented in table 2. It will be noted that race 4 was collected more often than all of the others combined, and that it occurred over wide areas of the United States. Of interest is the fact that in a study of physiologic races of *U. hordei* (5), the writer likewise isolated a widespread race (race 6), which occurred 114 times in 200 collections. This, like race 4 of *U. nigra*, also is characterized by the susceptibility of

TABLE 2.—*Frequency of occurrence and distribution by States of physiologic races of Ustilago nigra in 168 collections from 23 States*

| Location | Collections of race number | | | | | | | Total collections from the State |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Arkansas | 3 | ... | 1 | 1 | ... | ... | ... | 5 |
| Colorado | ..... | ... | ... | 4 | ... | ... | ... | 4 |
| Delaware | 1 | ... | ... | ..... | ... | ... | ... | 1 |
| Illinois | 2 | 2 | ... | ..... | ... | ... | ... | 4 |
| Indiana | 3 | ... | ... | ..... | ... | ... | ... | 3 |
| Iowa | ..... | 2 | ... | 9 | ... | ... | ... | 11 |
| Kansas | ..... | 1 | ... | ..... | ... | 1 | ... | 2 |
| Kentucky | 1 | ... | ... | ..... | ... | ... | ... | 1 |
| Maryland | 2 | ... | ... | ..... | ... | ... | ... | 2 |
| Minnesota | ..... | 2 | ... | 18 | 4 | 5 | 2 | 31 |
| Missouri | 2 | 1 | ... | 1 | ... | ... | ... | 4 |
| New York | 1 | 3 | ... | 11 | ... | ... | ... | 15 |
| North Carolina | 1 | ... | ... | ..... | ... | ... | ... | 1 |
| North Dakota | ..... | ... | ... | 2 | ... | ... | ... | 2 |
| Ohio | ..... | ... | ... | 1 | ... | ... | ... | 1 |
| Oklahoma | 1 | ... | ... | ..... | ... | ... | ... | 1 |
| Pennsylvania | 12 | 3 | ... | 1 | ... | ... | ... | 16 |
| South Dakota | ..... | ... | ... | 3 | 1 | 2 | ... | 6 |
| Tennessee | 3 | ... | 1 | ..... | ... | ... | ... | 4 |
| Texas | ..... | 1 | ... | 16 | ... | ... | ... | 17 |
| Virginia | 8 | ... | ... | 2 | ... | ... | ... | 10 |
| Washington | ..... | ... | ... | 2 | ... | ... | ... | 2 |
| Wisconsin | ..... | ... | ... | 15 | 2 | 7 | 1 | 25 |
| Total collections of each race | 40 | 15 | 2 | 86 | 7 | 15 | 3 | 168 |

Hannchen, Lion, and Odessa and by the resistance of Excelsior and Nepal[3] to its attack. Also, *U. nigra* races 1, 2, and 3 produced reactions similar to those of *U. hordei* races 8, 1, and 5, respectively, on the varieties common to both tests.

## PATHOGENIC STABILITY OF RACES

The data in table 1 show that the 7 collections typifying the races of *Ustilago nigra* were pathogenically stable throughout the years they have been studied. The remaining 161 collections tested in 1 or 2 years only, also produced such clear-cut reactions that there was no question concerning their race allocation. Two of three other collections tested, but not included here, were evidently mechanical mixtures of several races. The other, a

[3] Himalaya (C.I. 1312) was not used as a differential host in the earlier covered smut tests (5) but in recent years this variety has proved immune from *U. hordei* race 6, as well as *U. nigra* race 4.

collection from Missouri, may deserve classification as an eighth race of *U. nigra,* but the reaction of Excelsior to it has been variable.

## SUMMARY AND CONCLUSIONS

Seven distinct races of the seedling-infecting barley loose-smut fungus, *Ustilago nigra,* were found in 168 collections of that species from 23 States. The frequency of occurrence of the races and their distribution by States are tabulated.

Race 4 occurred more frequently than all the others combined. This race is identified by the same reaction of the differential varieties that identified a previously described race (race 6) of barley covered smut, which also occurred in over half of the collections of that smut in an earlier study.

In breeding for resistance against *Ustilago nigra,* as with other small grain smuts, the existence of physiologic races must be taken into account.

BUREAU OF PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND

## LITERATURE CITED

1. JOSEPHSON, L. M. Physiologic races in the fungus causing the intermediate loose smut of barley. (Abstract) Phytopath. 32: 11. 1942.
2. TAPKE, V. F. An undescribed loose smut of barley. Phytopath. 22: 869–870. 1932.
3. ————. A study of the cause of variability in response of barley loose smut to control through seed treatment with surface disinfectants. Jour. Agr. Res. [U.S.] 51: 491–508. 1935.
4. ————. Pathogenic strains in *Ustilago nigra.* Phytopath. 26: 1033–1034. 1936.
5. ————. Physiologic races of *Ustilago hordei.* Jour. Agr. Res. [U.S.] 55: 683–692. 1937.
6. ————. Occurrence, identification and species integrity of the loose smuts of barley, *Ustilago nuda, U. nigra* and *U. medians.* (Abstract) Phytopath. 31: 22. 1941.
7. ————. Occurrence, identification and species validity of the barley loose smuts *Ustilago nuda, U. nigra* and *U. medians.* Phytopath. [In press.]

## PHYTOPATHOLOGICAL NOTES

*An Unusual Sporophore of Trametes suaveolens Produced on Artificially Inoculated Wood.*[1]—In a preliminary study to determine the effect of environment on the gross morphology of fruit bodies and to compare those produced artificially with those found in nature, cultures were made as follows.

Two sections 7 inches long and 2½ inches in diameter were cut from fresh green branches of green ash, *Fraxinus pennsylvanica,* var. *lanceolata* Sarg., and soft maple, *Acer saccharinum* L. A hole 1¼ inches in diameter and 6 inches deep was bored in each and filled to within one inch of the top with a mixture of wheat and oats that had been autoclaved at 15 pounds' pressure for 1 hour on 2 successive days. Corks were placed loosely in the holes. The two pieces were then placed vertically in a gallon jar containing about 500 cc. of water, and the cover was screwed on lightly. The jar and contents were autoclaved for 1 hour at 15 pounds' pressure and allowed to cool in the autoclave.

When cool, the grain mixture was inoculated with a culture of *Trametes suaveolens* (L.) Fries isolated from a sporophore found on a maple stump near St. Paul, Minnesota, in November, 1940. Immediately after inoculation (Jan. 2, 1941) the holes in the wood sections were tightly plugged with corks and the jar cap tightened. The culture was kept in the laboratory.

After about 8 weeks mycelium was noticed growing out between the corks and the wood, and by May 1, a thick mycelial mat covered both pieces of wood. The quantity of water originally placed in the jar increased rather than decreased during this time, apparently being augmented by water released by the fungus during the decay process. A few rounded humps of mycelium formed near the upper end of the wood, but no definite fruiting primordia appeared. On August 13 the wood pieces, by this time bound firmly together by mycelium, were placed in a flat of moist sand beneath the north side of a greenhouse bench, the bottom ends being buried about 3 inches in the sand. Tap water was added to the sand occasionally. Although not exposed to direct sunlight, the specimens were located where the light was sufficient to permit the apparently normal growth of various green plants.

About September 1 shallow pores were noticed on the surface of one of the humps of mycelium, and by November 1 the compound sporophore (Fig. 1, A and B) had attained its final size, being approximately 4 inches wide. It was photographed, and agar cultures were made from it, on December 1, 1941.

The pores of the original fruit body are shown in figure 1, C, and those of the largest pileus in the compound sporophore produced in the greenhouse are shown for comparison in figure 1, D. Whether the obvious deviations from the normal in the general form of the sporophore and in the size,

[1] Paper No. 2031 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

shape, and position of the pores of the latter can be ascribed to the substrate on which the fungus grew or to light or humidity, we cannot say. The wood

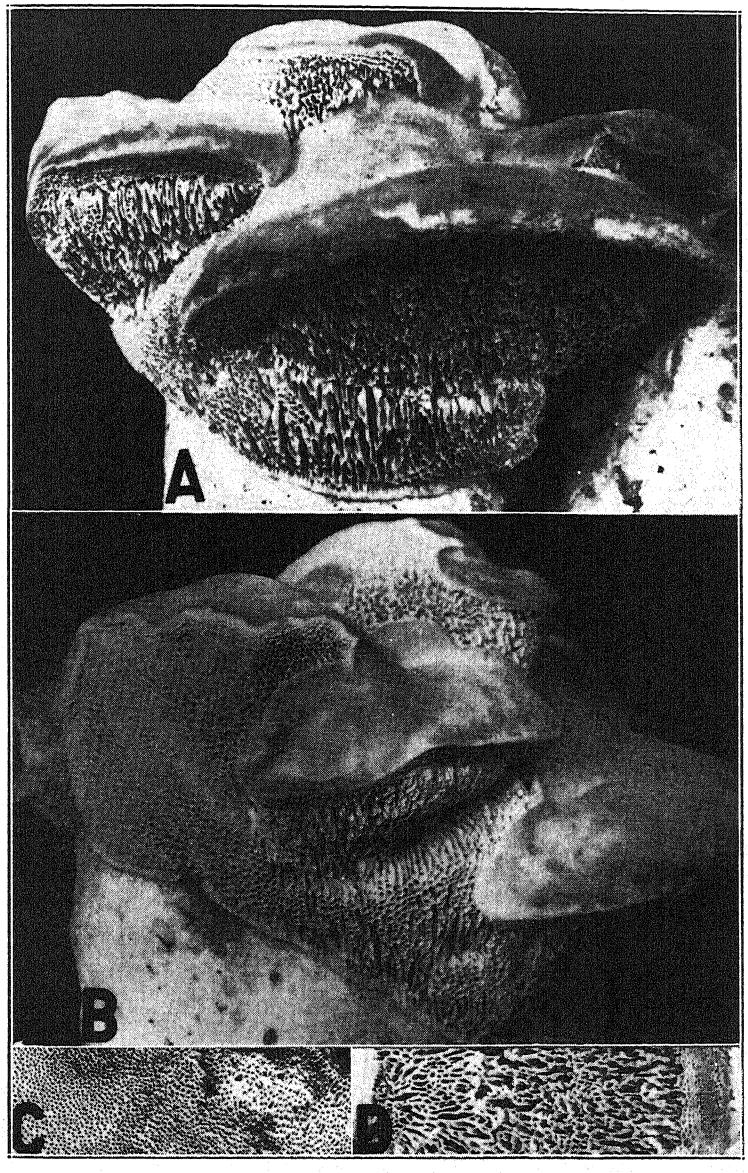



FIG. 1. A and B. Two views of the same clump of fruit bodies that developed on inoculated wood; note the horizontal pores. C. Pore surface of original fruit body. D. Pore surface of fruit body illustrated in A.

of both pieces was thoroughly rotted, but the grain also was almost entirely consumed. It would seem not an unreasonable assumption that the more

or less uniform diffused light may have played a part, since Christensen[2] reported a somewhat similar anomalous development of sporophores of *Daedalia confragosa* in diffused light in the laboratory. The fact that comparatively small differences in the environment can induce considerable variations in gross morphology of sporophores should make one wary of ascribing too much importance to minor morphologic differences between fruit bodies found in nature.—ELLIS F. DARLEY and CLYDE M. CHRISTENSEN, University Farm, St. Paul, Minn.

*A Method for Maintaining Phytomonas sepedonica in Culture for Long Periods Without Transfer.—Phytomonas sepedonica,* the organism causing ring rot of potato, may be considered a fastidious species and difficult to maintain on culture media. Even with Burkholder's special media no claims for longevity have been made. Stapp[1] failed to maintain it under any circumstances, even with repeated transfers for more than 18 months. The writer has found viability uncertain in transfers from agar cultures older than 30 days, kept under laboratory conditions.

Lumière and Chevrotier[2] maintained gonococci cultures several months when sealed with paraffin oil or vaseline. Michael,[3] employing solid media, extended the test to other organisms with good results. Bruni,[4] Parish,[5] and Birkhaug[6] kept a number of human bacterial pathogens viable under liquid paraffin for 8–24 weeks. These studies suggested the possibility of maintaining plant pathogens such as *Phytomonas sepedonica* under mineral oil; subsequent preliminary tests by this department indicated that the viability of the organism could be prolonged in this way. Approximately 2 years ago a number of fresh isolates of *P. sepedonica* were placed under sterile mineral oil and have since been tested for viability and pathogenicity.

Slants of Burkholder's media were inoculated with 16 cultures of *Phytomonas sepedonica.* Ten days later the cultures were covered with a layer of sterile mineral oil sufficient to extend well above the slant. Tubes were plugged with cotton and held at room temperature. To test viability at monthly intervals a 2-mm. loop was passed through the oil, a loopful of cells removed and streaked on a fresh agar slant. Before streaking, the loop was held against the inside wall of the test tube above the agar to remove much of the oil adhering to the cells. An effort was made to employ

[2] Christensen, C. M. Two cases of unusual development of fruit bodies. Mycologia **34**: 400–402. 1942.

[1] Stapp, C. Beiträge zur Kenntnis des *Bacterium sepedonicum* Spiekerm. et Kotth. des Erregers der "Bakteriumringfaul" der Kartoffel. Zeitschr. f. Parasitenk. **2**: 756–823. 1930.

[2] Lumière, A., and A. Chevrotier. Sur la vitalité des cultures de gonocoques. Comp. Rend. Acad. Sci. **158**: 1820–1821. 1914.

[3] Michael, M. Die Konservierung schwer. haltbarer Bakterienkulturen insbesondere des Gonococcus. Centralbl. f. Bakt. O. I. **86**: 507–510. 1921.

[4] Bruni, E. La conservazione in colture del meningococco. Ann. di Med. Nav. e. Colon. **36**: 396–398. 1930.

[5] Parish, H. J. Preservation of cultures under liquid paraffin. Jour. Path. Bact. **35**: 143–144. 1932.

[6] Birkhaug, K. E. Preservation of bacterial cultures under liquid paraffin. Science (n.s.) **76**: 236–237. 1932.

a uniform technique for each culture and each test. Readings were made after 14 days incubation.

Viability of cultures of *P. sepedonica* for the duration of this test and the age at which viability was apparently lost are indicated in Table 1.

Series B differs from Series A only in being placed under oil 3 months earlier than the latter. All were colony isolates from diseased tubers. After 10 months under oil most cultures were still viable and grew luxuriantly on removal from oil. After 18 months 25 per cent of the cultures were alive. In Series B a few were still viable after 17 months; however, after 21 months all were dead. The difference between individual cultures in ability to exist under oil was noticeable, certain ones losing viability after 6, others

TABLE 1.—*Summary of viability of Phytomonas sepedonica under mineral oil*

Series A

| | Number of months under oil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2 | 4 | 5 | 6 | 7 | 10 | 11 | 14 | 18 |
| Total cultures tested | 10 | 10 | 10 | 6[a] | 6[a] | 16[b] | 16 | 16 | 16 |
| Total cultures viable | 10 | 9 | 9 | 5 | 5 | 10 | 8 | 4 | 4 |
| Percentage of cultures viable | 100 | 90 | 90 | 83 | 83 | 62.5 | 50 | 25 | 25 |

Series B

| | Number of months under oil | | | | | |
|---|---|---|---|---|---|---|
| | 6 | 8 | 13 | 14 | 17 | 21 |
| Total cultures tested | 10 | 10 | 8[a] | 8 | 8 | 8 |
| Total cultures viable | 6 | 4 | 3 | 2 | 2 | 0 |
| Percentage of cultures viable | 60 | 40 | 37.5 | 25 | 25 | 0 |

[a] Some cultures lost by fungus contamination of oil.
[b] 10 additional cultures placed under oil at the same time as others but not previously tested for viability.

after 10, and some after 17 months. Cultures of *P. sepedonica,* viable after 18 months immersion, were tested for pathogenicity and induced typical ring-rot symptoms on leaf and tuber. Similar cultures without an oil covering will dry up and lose viability in 30–60 days.

Supplementary investigations employing other plant pathogens have demonstrated that *Phytomonas medicaginis* var. *phaseolicola* (halo blight of bean), and *P. phaseoli* (common blight of bean), under oil, retain their viability and pathogenicity at least 13–18 months. A like oil-immersion test applied to plant-pathogenic fungi indicates that the following remained apparently unharmed for at least 6 months (extent of test) and produced normal spore forms and mycelium when removed: *Fusarium eumartii, F. oxysporum, F. avenaceum, F. lycopersici,* and *Alternaria* sp. Further tests will be made at monthly intervals on all fungal and bacterial cultures to determine the viability end point.

It is believed this investigation has demonstrated the practicability of employing mineral oil for maintaining *Phytomonas sepedonica* and other phytopathogens for relatively long periods without transfer to new media.—ARDEN F. SHERF, Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska.

*On the Value of Spergon for Seed Treatment in Small-Grain Crops.*—Among organic compounds recently found valuable for seed treatment, tetrachloro-para-benzoquinone, main constituent of the dust fungicide Spergon, is of special interest. Its slight toxicity for animals and its low phytocidal action, compared with many other chemicals, are combined properties of considerable merit. This dust, first employed as a seed protectant for Lima beans and garden peas[1,2] promises a wider field of usefulness.[3,4] To determine its possible value as a fungicide for small-grain seeds, field tests were undertaken in Alberta in 1941. A few of the results of these tests are here reported.

TABLE 1.—*Relative effectiveness of Spergon, Ceresan, and formaldehyde in the control of covered smuts of wheat and oats*

| Treatment | Amount or concentration applied | Percentage smutted heads | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Edmonton | | Fallis | | Olds | | Castor |
| | | Wheat | Oats | Wheat | Oats | Wheat | Oats | Wheat |
| Check | None | 30.5 | 17.7 | 15.0 | 11.0 | 25.2 | 10.8 | 42.5 |
| Ceresan | ½ oz. per bu. | 2.2 | 0.0 | 2.2 | 0.0 | 0.2 | 0.2 | 2.0 |
| Spergon | 2 oz. per bu. | 0.3 | 7.0 | 0.0 | 1.2 | 1.0 | 11.5 | 0.2 |
| Formaldehyde | 1–320 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.5 | 0.0 |

Seed of wheat, oats, and flax was treated. The seed lots of the first two were artificially infested with their respective covered smut fungi and also carried a small amount of natural inoculum. In the case of flax, clean seed was used. Spergon was applied at the rate of 2 oz. per bu., a week before sowing. The other fungicides, tested comparatively, were applied according to usual recommendations at the rates indicated in the tables. Tests with flax were made at Edmonton and Castor, Alberta; the other grains were tested at Fallis and Olds, as well. At Edmonton, 6, elsewhere 4, replicates of each treatment and each check, randomized in blocks, were sown. The locations mentioned represent different soil types and climatic conditions. Castor is located in the dry open prairie in the brown-soil belt, Edmonton and Olds are in areas of higher rainfall in the black-soil park country, and Fallis in a wooded, gray-soil region. The same seed samples were used at all points.

[1] Cunningham, H. S., and E. G. Sharvelle. Organic seed protectants for Lima beans. Phytopath. (Abstract) 30: 4–5. 1940.

[2] Sharvelle, E. G., and B. F. Shema. A preliminary investigation of the value of a new seed protectant for canning peas in Minnesota. Phytopath (Abstract) 31: 20. 1941.

[3] Felix, E. L. Tetrachloro-para-benzoquinone, an effective organic seed protectant Phytopath. (Abstract) 32: 4. 1942.

[4] Leukel, R. W. Spergon as a seed disinfectant. Pl. Dis. Rptr. 26: 93–94. 1942.

Data on covered-smut control in wheat and oats are shown in table 1. The figures are averages of the replicates of each treatment. It will be noted that Spergon gave good control of covered smut or bunt of wheat at all points, comparing favorably in effectiveness with Ceresan and formaldehyde. In the case of oats, however, Spergon did not control covered smut as well as either Ceresan or formaldehyde.

The relative effects of Spergon and Ceresan on flax are indicated by average emergence and yield figures (Table 2). Both fungicides improved

TABLE 2.—*Relative effects of Spergon and Ceresan on the emergence and yield of flax*

| Treatment | Amount applied | Edmonton | | Castor | |
|---|---|---|---|---|---|
| | | Emergence | Yield | Emergence | Yield |
| | *Oz.* | *Per cent* | *Bu. per a.* | *Per cent* | *Bu. per a.* |
| Check | None | 53.0 | 8.4 | 59.8 | 5.5 |
| Ceresan | 0.5 | 81.5 | 9.3 | 71.1 | 6.6 |
| Spergon | 2.0 | 75.3 | 10.2 | 65.1 | 6.4 |

the emergence significantly at Edmonton, and Ceresan proved superior to Spergon (M.S.D. = 6). At Castor, a similar relationship held, though the difference between the fungicides was not significant. A tendency for both fungicides to improve yield is perhaps indicated by the consistently higher figures for the treated over the untreated seed lots. The differences between checks and treatments and between the treatments are, however, not significant. While yield response may be determined in part by emergence response, other factors may be even more important.

It may be concluded that Spergon has merit as a seed fungicide, especially for wheat and flax.—A. W. HENRY, University of Alberta, Edmonton, Alberta, Canada.

*Alternaria sp. on Grain Kernels Killed by High Temperature Storage.*—In connection with studies in 1941 on the effects of storage temperatures on germinability of certain seeds, it was observed that those of oats, wheat, and barley, stored 6 to 15 months at 105° F. were free from fungi; those from the same seed lots, stored at 36° and 50° F., were heavily infested, chiefly with *Alternaria* sp. Oat kernels stored 15 months at 36° F. (Fig. 1, A), wheat 6 months at 50° F., and barley 7 months at 36° F. (Fig. 1, E), developed abundant growth of *Alternaria* sp. during the regular seed-germination period of from 3 to 7 days. On kernels of oats, wheat, and barley from the same lots of seeds, stored for the same periods at 105° F., the *Alternaria* sp. had been killed and, on germination, the seeds were free from the fungus (Fig. 1, B, F). All the seeds were germinated on moist blotters in germinating dishes.

The seeds on which the above observations were made were received as follows: Oats from Aberdeen, Idaho, August 16, 1939; wheat from Lincoln,

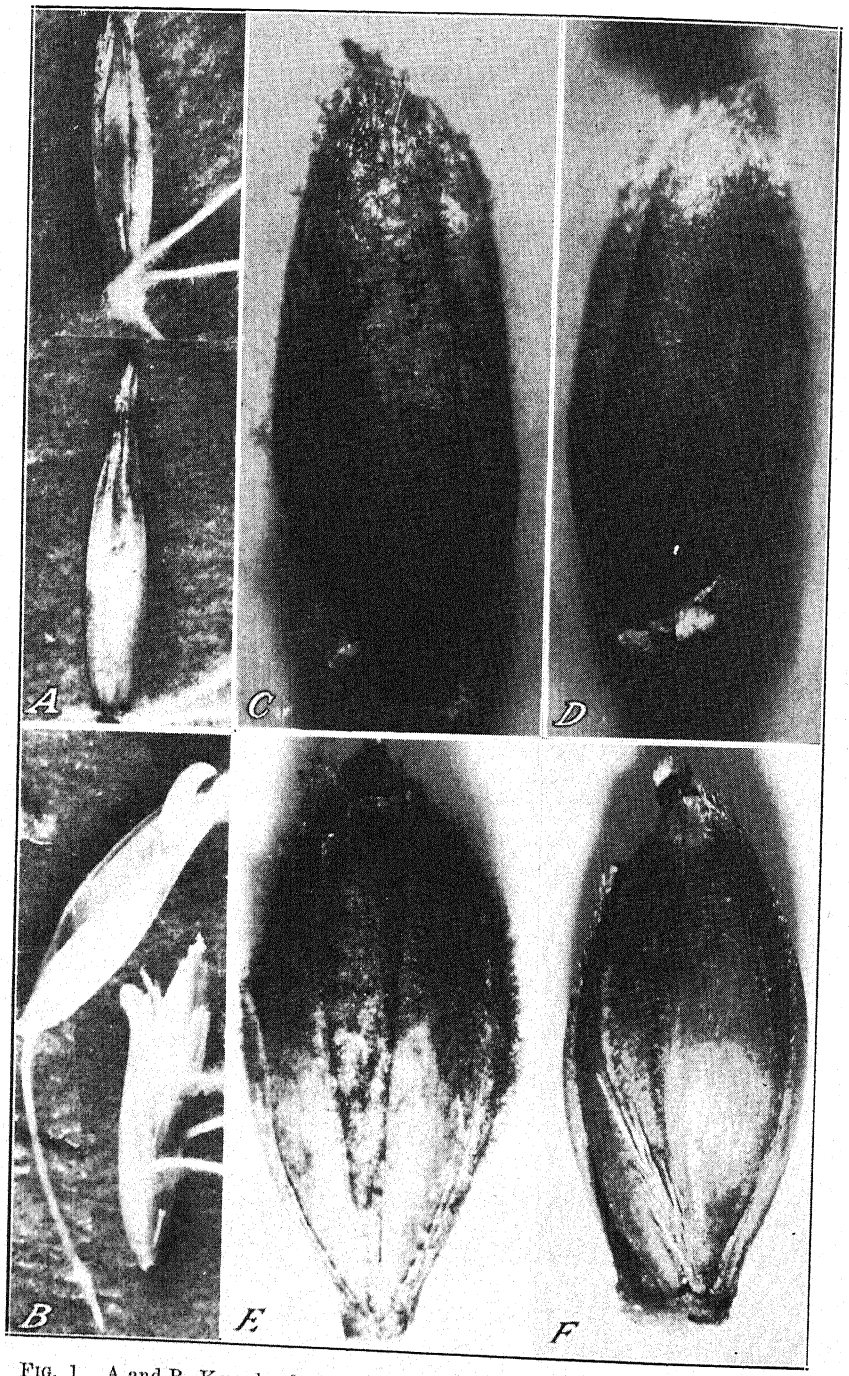



FIG. 1. A and B. Kernels of oats stored 15 months: A, at 36° F.; B, at 105°. C and D. Wheat stored 7 months: C, at 50°; D, at 105°. E and F. Barley stored 7 months: E, at 36°; F, at 105°. All germinated on moist blotters in germinating dishes. A, C, and E, stored at the lower temperatures, showed abundant development of *Alternaria* sp.; B, D, and F, stored at 105° F., were free from the fungus. In C to F, the primary roots were clipped off.

Nebraska, July 2, 1940; and barley from Arlington Farm, Virginia, June 21, 1940. In each case when the grains had been stored 6 months or longer at 105° F., the fungus originally on the kernels was killed; in the samples stored at 36° and 50° F., the fungus was not killed.

A test was made to confirm the above observation. The seed used was wheat received from Lincoln, Nebraska, July 2, 1940. One sample of the wheat had been stored 7 months at 50° F. and another for the same period at 105° F. Eight kernels from each sample were placed on moist blotters in germinating dishes under sterile conditions. After 7 days incubation at 68° F., the kernels, all of which had germinated, were examined for the presence of *Alternaria* sp. The wheat kernels that had been stored 7 months at 50° F. were heavily infected with *Alternaria* sp.[1] (Fig. 1, C), while those stored for the same period at 105° F. were free from the fungus (Fig. 1, D). —EDGAR BROWN AND ALICE L. ROBERT, Bureau of Plant Industry, U. S. Dept. of Agr., Washington, D. C.

*Stem Rust on Triticum timopheevi.*[1]—*Triticum timopheevi* Zhuk. has been highly resistant to practically all physiologic races of stem rust, *Puccinia graminis tritici*, with which it has been inoculated. But race 189, an unusually virulent race in Peru, can attack seedlings and adult plants of *T. timopheevi*;[2] and race 19, identified more often than any other race in collections from experimental field plantings of *T. timopheevi* in the United States, has been moderately virulent on seedlings and on older plants inoculated in the greenhouse by the writer and by Stakman and his co-workers.

In 1942 there was 35 per cent of stem rust on *Triticum timopheevi* in certain experimental plots at University Farm, St. Paul, Minnesota. All plant parts were rusted, from the lowermost leaf sheaths to the glumes, awns, and peduncles. The reaction indicated moderate susceptibility, although most pustules were small and surrounding tissues were slightly discolored (Fig. 1, A). Wheat varieties and hybrids in the experimental plots were exposed to stem-rust inoculum early in the season, border rows between plots being inoculated as early as May 26, by means of a hypodermic syringe, with water suspensions of urediospores of six different physiologic races (15B, 17, 34, 36, 56, 147) of *P. graminis tritici*. By June 3 inoculum was spreading from rusted borders to the experimental plants. Inoculations every 5 to 7 days provided a new and an ever-increasing supply of rust in the borders for a natural spread to the experimental plants, which themselves never were inoculated directly.

Seven collections of stem rust were taken from various replicates in the

[1] The *Alternaria* on the wheat kernels was identified as belonging to the *A. tenuis* group, as interpreted by Elliott, J. A., Am. Jour. Bot. 4: 472. 1917.

[1] Published as Paper 2039 in the Journal Series of the Minnesota Agricultural Experiment Station. Assistance in the preparation of these materials was furnished by the personnel of Work Projects Administration, Official Project No. 265–1–71–236, Subproject No. 491.

[2] Garcia-Rada, G., J. Vallega, W. Q. Loegering, and E. C. Stakman. An unusually virulent race of wheat stem rust, no. 189. Phytopath. 32: 720–726. 1942.

3 different plantings of *Triticum timopheevi*. Upon identification every collection consisted of race 15B, apparently the only one of the 6 races furnished in the inoculum that was virulent on this species of *Triticum*. Newton, Johnson, and Peterson[3] reported on the seedling reaction of *T. timopheevi* to 20 physiologic races of *Puccinia graminis tritici*, among which was race 15. The species was highly resistant and from the work of Stakman and Loegering[4] it seems that the Canadian race 15 was different from the race

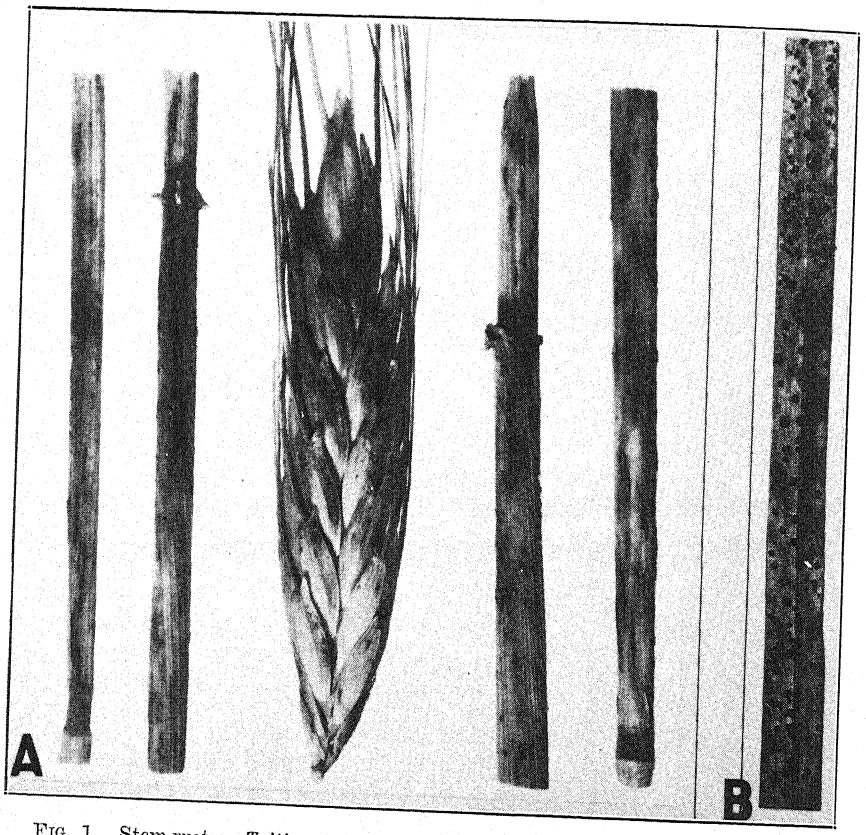



FIG. 1. Stem rust on *Triticum timopheevi* Zhuk. A. Telia of stem rust on culms and head of *T. timopheevi* grown in the field at St. Paul in 1942. The stems were boiled in sodium hypochlorite before they were photographed, and most of the hairs that characterize the host species were broken. ×about 2. B. The susceptible reaction of seedlings of *T. timopheevi* to race 15B of *Puccinia graminis tritici*. ×about 2.

15B used in the present work. Seedlings of *T. timopheevi* were susceptible to race 15B and gave a type 3+reaction (Fig. 1, B). Loegering and Stakman[4] reported that race 15B is virulent on Rival, a newly recommended variety of hard red spring wheat, whereas race 15A does not attack it. They pointed out the danger of such a biotype of race 15 in the spring wheat area

[3] Newton, Margaret, T. Johnson, and B. Peterson. Seedling reactions of wheat varieties to stem rust and crown rust. Can. Jour. Res. (C) 18: 489–506. 1940.

[4] Loegering, W. Q., and E. C. Stakman. Biotypes within *Puccinia graminis tritici*, race 15. (Abstract) Phytopath. 32: 12–13. 1942.

and found that both types of the race are present in the United States. It now seems probable that *Triticum timopheevi* and its hybrids also will be endangered by race 15B if that race of rust becomes widespread and destructive in wheat-growing areas and if inoculum is abundant and conditions favor rust infection for a protracted period. This is merely another illustration of the fact that there are few species of wheat or wheat allies that are universally resistant to rust.—HELEN HART, Agricultural Experiment Station, University Farm, St. Paul, Minn.

# BOOK REVIEW

BALDWIN, HENRY IVES. *Forest Tree Seed.* Chronica Botanica Co., Waltham, Mass.; G. E. Stechert and Co., New York City. 240 pp. 28 figs., 1942. $4.75.

The author has selected and compiled considerable technical and practical knowledge on tree seed. Although the book is of value to all engaged in handling various kinds of seed and studying different aspects of the whole field of seed problems, "Forest Tree Seed" will be most helpful to silviculturists and especially so to those interested in the conservation and reforestation of timberlands.

The subject matter is divided into 20 chapters. The information presented centers around such topics as development, structure, and production of tree seed; some of the other phases of the seed problems that are presented include origin, collection, extraction, and germination of seed, internal and environmental factors affecting germination, and seed viability and stimulation. Chapter 7, Biotic enemies of tree seeds, contains some sections especially interesting to the pathologist. They are as follows: Fungi, Seed-borne diseases, Diseases of cones, Seed disinfection and antisepsis, Physical treatments, Chemical treatments, and Aseptic germination. Each topic is developed by a résumé of most of the pertinent literature with significant specific details being given in small type. Reference lists, most of which are extensive, are to be found at the end of all but the last chapter, which consists of a glossary of tree-seed terms and subject and author indices.

The outstanding contribution of the book is that it brings together and makes readily available a considerable amount of knowledge heretofore to be found only in unassembled publications.—WILLIAM C. DAVIS, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

# WAR COMMITTEE OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY—REPORT OF MEETING IN COLUMBUS, OHIO, FEBRUARY 13 AND 14, 1943

The committee met in conjunction with a meeting of the officers and the Council of the Society called in place of the cancelled annual meeting. The Plant Protection Committee of the National Research Council met with the War Committee. E. F. Phillips, chairman of the War Committee of the Association of Economic Entomologists, and Neil Stevens, chairman of the War Committee of the American Mycological Society, were present as representatives of their respective committees. The following members of the War Committee were present: C. C. Allison, G. M. Armstrong, H. P. Barss, F. J. Greaney, R. J. Haskell, J. G. Horsfall, L. M. Hutchins, R. S. Kirby, I. E. Melhus, J. C. Walker, and J. G. Leach (Acting Chairman in the absence of E. C. Stakman).

All day Saturday was spent hearing and discussing the reports of regional committees, sub-committees, and members at large. At a final meeting held late Sunday afternoon, a number of resolutions were adopted. These appear at the end of this report.

Space will not permit a complete report of Saturday's meeting. A more detailed report has been mimeographed and sent to the contact men in each State. Some of the more significant items reported were as follows:

Attention was called to occupational Bulletin No. 23 as amended December 14, 1942, authorizing draft boards to defer not only full-time instructors and research workers in agricultural sciences, but also part-time research assistants if working on projects certified to be related to the war effort.

Local Board Release No. 159, authorizing the establishment of special committees on manpower in critical scientific fields, was discussed. The Executive Committee has requested G. W. Keitt to make a survey of the situation in Plant Pathology to determine if such a committee is desirable. *It was voted to defer submission of the resolution on manpower, drawn up by the Upper Mississippi Valley Group at its Chicago meeting, until after this survey is completed.*

A memorandum, prepared by E. C. Stakman, and submitted to Governor Lehman, Director of the Office of Foreign Relief and Rehabilitation Operations, by Ross G. Harrison, Chairman of the National Research Council, was explained briefly. This memorandum recommends that people in the countries occupied by American armed forces be helped to help themselves through increased efficiency in agricultural production. It is further recommended that men with specialized training in agricultural sciences, already in the armed forces, be detailed by the Army to cooperate with other authorities in the direction of such a program.

The importance of regional meetings to consider problems of mutual interest in the war effort was next discussed. In view of some difficulties already experienced by workers in some States in receiving authorization to attend regional meetings of the War Committee, *the Executive Committee was instructed to write a suitable letter to the Directors of Experiment Stations and Directors of Agricultural Extension, soliciting their interest in, and encouragement of, regional meetings during the war period.*

*Dr. Stakman's recent suggestion that quarterly reports of the War Committee be published in Phytopathology was discussed and approved by the Committee.*

*A suggestion that the name of the Committee be modified by eliminating the word "Emergency" was approved.*

F. J. Greaney told of the manpower situation in Canada, where all universities are under complete military control, and where plant pathologists are being called upon for medical aid on the home front.

J. C. Walker reported on a program of bean-seed certification for control of bacterial blight, being developed by L. H. Person of Louisiana. He also stressed the critical shortage of many vegetable seeds and the importance of seed-treatment in seed conservation.

E. F. Phillips reported on manpower situation in Entomology, pointing out that the Army is making good use of the technical training of entomologists, practically all entomologists inducted into the service being assigned to duty as entomologists, but that the demand of the Army for entomologists is increasing steadily, and has already reached the point where the effectiveness of entomology in the food-production plan is being threatened.

J. G. Horsfall reported briefly on coordinated research on fungicide dosage and substitute fungicides. More complete reports will be mimeographed and distributed.

R. J. Haskell reminded the committee of his offer to distribute informational material to 130 contact men through the country, and stressed the importance of more adequate exchange of information.

Harry O'Brien led an inspiring discussion on problems of publicity. He gave us many valuable suggestions and promised to help us as much as possible.

At the final meeting, called at 3:00 o'clock, Sunday afternoon, the following resolutions were adopted:

1. That a nation-wide seed-treatment campaign be declared for the reduction of plant-disease losses of the crops essential in the war-production program with special emphasis on oats, barley, wheat, sorghum, flax, corn, cotton, peanuts, and certain vegetable crops as a means of insuring against poor stands and as a means of stretching limited seed supplies.

2. That efforts be made to include established plant-disease-control recommendations into the action programs for achieving war-crop goals.

3. That plant pathologists in each State be urged to make every effort to obtain information on the development of major disease hazards throughout the season, with the aid of such other agencies and individuals as may be available, with a view to the issuance of timely warnings to growers and recommendations of immediate measures to be taken to check epidemics, or to lessen losses that would otherwise result.

4. That the Seed-treatment Committee of the Society be asked to prepare 1943 recommendations for the seed-treatmnet of wheat, oats, barley, sorghum, flax, and corn for distribution to all States.

5. That the Seed-treatment Committee be encouraged to work with seed producers and seedsmen with a view toward getting more seeds treated prior to distribution and sale, and/or getting directions for treatment printed on packages, or packets and in seed catalogs.

6. That efforts of the Seed Certification Committee to obtain the treatment of certified seed be endorsed by the War Committee.

7. That the Fungicide Committee of the War Committee be asked to distribute promptly a condensed statement of their present opinions as to measures for conserving fungicides.

Respectfully submitted,
J. G. LEACH
*Acting Chairman*

# BLISTER RUST RELATIONS OF CULTIVATED SPECIES OF RED CURRANTS

GLENN GARDNER HAHN

(Accepted for publication September 8, 1942)

## INTRODUCTION

In 1922, when Spaulding (12) reported blister-rust-inoculation studies of a considerable number of red and white garden currants, performed by himself and associates, there were practically no data in the literature dealing with this phase of research on *Cronartium ribicola* Fisch. These ribes were grouped by him (12, Table 1) for convenience under the name *Ribes sativum* (Rchb.) Syme (*R. vulgare* Lam.), as it was impossible at that time to assign them to the proper species to which they individually belonged. Shortly thereafter Thayer (13) published the results of taxonomical and field studies of the red and white currants. He found that they were of mixed parentage, but concluded that certain varieties sprang from each of the following species: *R. petraeum* Wulf., *R. rubrum* L., *R. sativum,* and *R. sativum* var. *macrocarpum* Bailey.

Before entering into a discussion of the known facts concerning the pathological relationships of the different groupings of the cultivated species of red currants according to the Thayer (13) classification, and an estimation of pathological data as they relate to white-pine infection studies, the results of recent greenhouse investigations of Red Dutch (Holländische Rote) are presented. This commercial variety of continental Europe, which has been in cultivation many years, is compared morphologically with another variety of similar origin, the commercial variety Viking (syn. Rød Hollandsk Druerips) from Norway. Viking is the only resistant type of red currant variety reported in blister-rust literature that has been investigated intensively (1, 4, 6, 7, 8).

In this paper the anglicised name "Red Dutch" is used for the continental European variety Holländische Rote (syn. Rouge de Holland). As Thayer (13) has pointed out the terms "Dutch" and "Holland" are unfortunately not synonymous, and the "Red Dutch" of the continent is different from the "Red Dutch" of England and the "Red Dutch" of the United States. The varieties from England and the United States belong to *rubrum* and *sativum,* respectively, whereas the continental variety shows predominant *petraeum* characteristics.

## RED DUTCH (HOLLÄNDISCHE ROTE)

### Pathological History

At the time the writer was performing greenhouse inoculation tests with the blister-rust-immune Viking at the Royal Botanic Garden, Edinburgh, Scotland (4), and in the United States (6), the late, eminent forest pathologist, Professor von Tubeuf, assisted by Wolpert (15, p. 436), was conduct-

ing, independently, field inoculation tests with another rust-immune garden variety, Red Dutch (Holländische Rote), at Munich, Germany. The last-named variety has been the subject of pathological investigations by other European scientists, who reported it as being highly resistant to *Cronartium ribicola*. As early as 1903, Red Dutch was recognized by Ewert (3) as being exceedingly resistant to the disease of white pines. Twenty years later Schellenberg (9), in Switzerland, made a similar report as the result of an investigation of the ribes population in his country, studied for the purpose of determining the individual significance of the different species in the spread and intensification of white-pine blister rust. In Switzerland, Red Dutch has been cultivated extensively as a commercial variety, and observations could be made readily in localities where it grew in close proximity to the commonly cultivated and heavily infected European black currant (*Ribes nigrum* L.).

Similarly, in 1917, Tubeuf (14, p. 300) reported, on the basis of field observations, that the variety Red Dutch (Holländische Rote) was not attacked by blister rust. Susceptible ribes in the immediate vicinity of this variety, however, were severely affected by the disease. It was not until 1928 to 1932 that he and Wolpert (15) demonstrated Red Dutch to be immune from blister rust as the result of inoculation tests covering five years. Shortly thereafter Tubeuf (16, 17) advanced recommendations for the control of white-pine blister rust in Germany, wherein he advocated, along with the complete destruction of the damaging European black currant, the cultivation solely of the rust-immune Red Dutch as a substitute for all other horticultural varieties, and its distribution under controlled conditions.

## Inoculation Results

In 1937, G. M. Darrow, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, became interested in the rust-immune red currant reported by Tubeuf as the result of information given to him by the writer. The following year at Darrow's suggestion, cuttings of the variety were obtained from the Grafrath Experiment Station, Munich, by the Division of Plant Exploration and Introduction (P.I. No. 127062) for study in this country. It is not known whether this stock originated from the same clone as that investigated by Tubeuf (15). We are probably correct, however, in assuming that the introduced Red Dutch stock represented the variety as it is distributed through trade channels on the continent.

After the receipt of the import stock, part of the shipment was retained at Beltsville and Glenn Dale, Maryland, for propagation, and 50 cuttings were sent at a later date to the writer at New Haven. Unforunately the cuttings arrived in poor condition and the writer succeeded in obtaining only two plants from the entire lot. The number of experimental plants at New Haven was augmented later by four additional plants obtained in 1941 through the courtesy of W. E. Whitehouse, Division of Plant Exploration and Introduction, Bureau of Plant Industry.

*On Red Dutch.* On June 5, 1939, 2 potted plants of Red Dutch, growing vigorously in the greenhouse, and with leaves showing all stages of development from those unfolding at the growing tip to those fully mature, were tested with freshly collected aeciospore inoculum[1] of *Cronartium ribicola* collected in Maine. A total of 31 leaves were inoculated and, approximately 2 weeks later, the same leaves on these 2 plants were reinoculated with aeciospore inoculum collected in Connecticut. In these tests the European variety proved immune.[2] The following year, on June 21, the same Red Dutch plants were retested with aeciospore inoculum collected in Maine. A total of 55 leaves were inoculated and the same results were obtained as in 1939.

On June 2, 1941, the experiments on the same two Red Dutch plants were repeated with Connecticut aeciospore inoculum. A total of 80 leaves were tested and these plants for the third season proved to be immune. At the same time 30 leaves on 2 of the Red Dutch plants received from Glenn Dale also were tested, with similar results. Later, on June 19, the two remaining plants (29 leaves) from Glenn Dale were tested with urediospore inoculum produced on heavily infected European black currants growing in the greenhouse. Red Dutch proved to be immune also from urediospore infection.

In the above experiments, it was observed that mature leaves of Red Dutch inoculated with *Cronartium ribicola* were unaffected. However, tender leaves that had just become fully expanded or were in the process of becoming so showed the presence of necrotic flecks. Similar flecking has been described and illustrated by the writer (4, Pls. 13, 14) on young leaves of Viking inoculated with blister-rust spores. Anderson (1) demonstrated cytologically that in Viking, these necrotic flecks were the result of the invasion of the new leaves by the blister-rust-spore germ tubes, which extended into the substomatal cavities for a brief distance, only to degenerate and die shortly after penetration. He also showed how the death of the invading germ tubes was associated with the death of host cells in their immediate proximity. Although a similar cytological study of probable germ-tube penetration has not been made in the case of Red Dutch (Holländische Rote), it is reasonable to expect that what occurs in the case of Viking also takes place in the case of Red Dutch. Red Dutch has been under observa-

[1] The writer desires to express his appreciation to Messrs. J. M. White, J. E. Riley, Jr., and Alton Miller, Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, for their cooperation in the collection of blister-rust aeciospore inocula used in the experiments outlined in this paper.

[2] The term "immune" has been applied to the Viking currant in previous publications (4, 6, 7, 8). As the writer indicated previously, the Norwegian variety at certain stages of leaf development is not free from the invasion of germ tubes of *Cronartium ribicola.* However, Anderson (1) has demonstrated clearly by cytological studies, which have corroborated extensive observations made by European pathologists and the writer, that *the infectious agent is unable to establish itself in Viking, i.e.*, there occurs the prompt death of both the invading pathogen and the host tissue itself at the point of attack. Such a condition may be described as "abortogenic necrosis," to use a term submitted at a meeting of The American Phytopathological Society (18). On this basis the writer has reserved the term "immune" for the case of Viking and other red currant varieties showing a similar response to an invasion by *C. ribicola.*

tion by European pathologists *for many years* and fructifications of blister rust on leaves of this common European variety have never been reported.

In every experiment *Cronartium ribicola,* inoculated on known-susceptible ribes used as "checks" to establish the viability of the inoculum and suitability of the environmental conditions, produced abundant fruiting bodies. During all of the tests urediospores of blister rust formed on these and other susceptible ribes, including heavily infected European black currants, were being disseminated naturally during the summer and autumn in the greenhouse in which the tests were carried out. Although the Red Dutch plants under investigation were exposed constantly to urediospore inoculum, they maintained their immunity from natural infection.

*On Viking Selfed-seedling "Escapes."* The experiments described above included tests performed simultaneously with two naturally pollinated, second-generation seedlings of the rust-immune Viking (8). These plants undoubtedly originated as the result of self-pollination for there were no other red currant varieties growing nearby, and there is no evidence that pollen of currants is carried any appreciable distances. Accordingly they could be regarded as Viking selfed-seedling "escapes," since they appeared spontaneously in a small field planting of the red currant from Norway maintained for horticultural testing. These two escapes showed the morphological characteristics of the Viking parent.

In 1939, 24 leaves of the Viking escapes in all stages of leaf development were tested with Maine aeciospore inoculum, and later, reinoculated with Connecticut aeciospores. In both tests the selfed seedlings proved immune. Similar results were obtained on the same plants in 1940 and 1941, when 67 leaves were tested, 25 with Maine aeciospore inoculum and 42 with Connecticut aeciospore inoculum. As in the case of the Red Dutch experiments, although the Viking escapes were exposed constantly to free urediospores of *Cronartium ribicola* produced abundantly in the greenhouse on European black currants, they continued immune from the pine disease.

## Comparison with Viking

Tubeuf (15, p. 439) classified Red Dutch (Holländische Rote) as the hybrid species, "*Ribes houghtonianum* Jancz. (= *R. rubrum* × *R. sativum* or *R. pallidum* Otto and Dietrich)." In a footnote (15, p. 460), however, he called attention to the taxonomic opinion of the well-known American horticulturist, Alwin Berger (2), who regarded "Holländische Rote, Rouge de Hollande, German Sour, Verrières rouge, etc." as synonyms of the hybrid species, "*R. pallidum* (= *R. petraeum* × *R. rubrum*)." It is here of interest to point out that both Thayer and Berger recognized Red Dutch as having originated as a *petraeum* hybrid.

The name Red Dutch, which, according to Thayer (13), is one of the oldest garden currants known, first appeared in print in 1670 or 1690. According to the opinion of European horticulturists it originated in part from the high mountain species, *Ribes petraeum, i.e.,* Ewert (3) quotes Maurer of

Jena who was of this opinion. The rust-resistant Red Dutch reported by Schellenberg (9) was undoubtedly of the same origin. Concerning the identity of the Red Dutch, grown commonly in Switzerland, the writer received the following information contained in a written communication obtained through the courtesy of Professor Gäumann of Zurich from Dr. K. Kobel, Horticultural Experiment Station, Wädenswil, (translation) "The currant variety 'Holländische Rote' is widely distributed in Swiss nurseries. This doubtless is true also of the German ones. It is the most important of the cultivated varieties of *Ribes petraeum* to be found abundantly in both countries. . . . It is very probable therefore that the 'Holländische Rote' of Tubeuf and Schellenberg's variety of the same name are identical." The writer (6) already has pointed out that Viking, according to an authoritative Norwegian source, originated as a cross between *R. petraeum* × *R. rubrum*. It becomes very evident, therefore, that the rust-immune varieties, studied pathologically and reported by Ewert, Schellenberg, Tubeuf, and the writer, respectively, were all derived in part from *R. petraeum*, and are closely related.

A comparative morphological study made by the writer (8) of Viking and the hybrid species, *Ribes pallidum*, growing in the Ribes Collection maintained at the Arnold Arboretum, Harvard University, and later a similar study made of Viking and vigorous potted plants of Red Dutch growing in the greenhouse, demonstrated that all of these produced foliage on the new shoots, which showed *petraeum* characteristics, *e.g.*, leaves with three prominently pointed lobes, the lateral ones often unequal in size, the right lobe being frequently the larger (6, Pl. 1; 8, Fig. 1). As has been reported for Viking (6), growth was vigorous, and the unequally lobed leaves were longer than broad, dark green, glossy, rugose, and pale and slightly pubescent beneath. New leaves were held stiffly upright in a conspicuous cupped-shape manner by stout petioles tinged with red. Both Ewert (3), describing Red Dutch, and Darrow (6, p. 3), describing Viking, pointed out that these closely related varieties could be differentiated readily from other garden currants.

In a recent publication (8) it was stated that leaves of naturally pollinated second-generation seedlings of Viking, which for all practical purposes could be regarded as selfed (in seed formation, although cross-pollination with susceptible red currants was not excluded absolutely, its occurrence was most unlikely), for the most part resembled those of the Viking parent. Several hundred of these rust-immune seedlings have been grown in the field since 1936 under conditions of favorable cultivation at the Marsh Botanical Garden, Yale University, and at the Connecticut Agricultural Experimental Farm, Mt. Carmel, Connecticut.[3] Mature plants thus derived from Viking seed showed morphological characters of bush, leaves, and flowers that gen-

[3] The writer wishes to express his appreciation to W. L. Slate, Director, and W. R. Singleton, geneticist, Connecticut (State) Agricultural Experiment Station for courtesies extended and for their cooperation in making it possible to carry on field tests with Viking seedlings at the State Experimental Farm.

erally resembled the *petraeum* hybrid whence they came. Some of these, however, produced white fruit and on bushes whose growth characters resembled closely those of bushes producing red fruit. A number of selections of red- and white-berried plants have been made from the two field plantings. There are excellent chances that a testing of selfed seedlings of these second-generation progeny will demonstrate some of them to be homozygous, *i.e.*, breed true for rust immunity.

The same field plot, in which selected rust-immune Viking seedling stock is being tested, also contains plants of Red Dutch. The fruitful (6) Norwegian commercial variety has shown itself to be well suited to the northeastern part of the United States. The writer is of the belief that the Red Dutch from Germany will be found adapted likewise to this part of the country.

## DISCUSSION OF RUST RESISTANCE SHOWN BY HYBRID GROUPS OF RED CURRANTS

### Ribes petraeum Group

In the foregoing the rust resistance of Red Dutch (Holländische Rote) and Viking (syn. Rød Hollandsk Druerips), two representatives of a small group showing markedly *Ribes petraeum* characters, has been considered. Accumulated pathological data on other members of this group have shown them to be not only very highly rust-resistant but also some of them, as in the case of Red Dutch and Viking, to be immune from the pine disease. These resistant varieties include Prince Albert (syn. Rivers Late Red, Rivers) and Long Bunch Holland (syn. Holland, Franco-German).

According to Thayer (13), both Long Bunch Holland and Prince Albert are resistant not only to disease and insect attack but also to heat and drought in the Prairie States. In that section of the country he stated that the former variety had done very well, and cited another horticulturist, J. L. Budd, who early (1880) reported it as being the only variety then known that preserved health of leaf during dry, hot summers in this country. Budd had observed that when most of the leaves had fallen from other currant varieties, leaves of Long Bunch Holland were still green and unaffected by leaf diseases. The observations of Budd and Thayer (13) in the United States are in agreement with European observations. Tubeuf (14, p. 300; 15, p. 458), in emphasizing the fact that Red Dutch (Holländische Rote) was not attacked by blister rust, also stated that its foliage was free from *Gloeosporium ribis* (Lib.) Mont. and Desm. infection, as well as from insect damage, statements that were in agreement with similar ones made previously by Ewert (3).

The garden currant blister-rust-inoculation experiments reported by Spaulding (12) included a number made on several *Ribes petraeum* hybrids —Prince Albert, Franco-German, Holland, and Rivers. On the first-named variety Spaulding recorded 18 tests, 4 of which succeeded and resulted in a *slight* infection, both in the greenhouse and out-of-doors. On the remaining varieties tested, he failed to obtain fruiting bodies as the result of 21

tests on Franco-German, 5 on Holland, and 4 on Rivers. Infection on leaves of Prince Albert was not qualified by stating the degree of uredium and telium production, so we have no way of knowing the amount of telia actually formed. We do know however that on the most susceptible variety of the *petraeum* group tested, fruiting bodies were exceedingly scant.

In 1930 the writer (5) reported additional experiments on *Ribes petraeum* stock of the varieties Prince Albert, Franco-German, and Holland. The Holland stock was authenticated by and received from Thayer (13) and G. M. Darrow. The writer found, as Spaulding (12) had reported previously, that Prince Albert bore only a scant infection, which produced very few uredia, whereas the other two forms were immune from *Cronartium ribicola.* Recently the writer received a report of blister-rust susceptibility tests being conducted in currant and gooseberry plantations at the Central Experimental Farm, Ottawa, Canada. The Canadians have found both Viking and Franco-German to be immune from *C. ribicola* over a period of 6 years, 1935 to 1940, despite the fact that these varieties were growing near infected European black currants that became heavily infected during certain years.[4]

Currants derived from *Ribes petraeum* are so highly resistant to blister rust that one is tempted to speculate on the exact form from which these commercial varieties originated. According to Thayer (13), who has as his authority the eminent ribes taxonomist, Janczewski, the probable form of *petraeum* to which we are indebted for our disease-resistant varieties of this species was *R. petraeum bullatum* Janez. It is here of great interest to note that among the rust-resistant, second-generation Viking seedlings (8) referred to above, there occurred a small percentage of plants producing leaves that were exceedingly dark green, smaller (as compared with Viking), and pronouncedly bullate. It would be interesting to compare this particular form morphologically with Janczewski's variety.

Despite the fact that *Ribes petraeum* has been reported susceptible to blister rust (12, 15), there undoubtedly are forms of it that we know very little about in this respect, *i.e.,* we do not have any information on the rust susceptibility of *bullatum,* and data on this variety would be extremely valuable. According to Budd, who is cited by Thayer (13), the variety Long Bunch Holland probably originated from northern European *petraeum* stock. In connection with this opinion, it might be worth while to draw attention to recent correspondence (1938–1939) carried on by the writer with Dr. K. Lepik, Phytopathological Experiment Station of the University of Tartu in Esthonia, concerning blister rust infection of *Ribes petraeum.* Lepik replied that the form in his country became only slightly infected. He submitted representative specimens of *Cronartium ribicola* (telia) on leaves of *R. nigrum* and *R. petraeum;* the former showed 100-per cent infection, whereas the latter showed approximately 5 per cent of

[4] Statements concerning blister-rust-horticultural investigations at the Central Experimental Farm, Ottawa, were communicated through the courtesy of that Station and Mr. D. S. Blair, Assistant in Pomology.

the total surface of mature leaves with telia at the time of collection at the end of August, and the fruiting bodies occurred mostly on necrotic leaf tissue.

In both Red Dutch (Holländische Rote) and Viking, the factor for rust resistance is undoubtedly dominant, but we do not know whether the former is homozygous for resistance. We do know, however, that Viking (8) is nearly so, for, considering the very small percentage (3.7 per cent) of susceptibles that appeared among a large population (1835 seedlings) of naturally pollinated Viking seedlings studied, the indications are that probably only a very small percentage of them are heterozygous, whereas the majority must be homozygous.

Since 1937 the writer has continued to carry on the greenhouse investigation of the susceptibility of the few nonimmune Viking seedlings, appearing in the tests referred to above, in order to determine the degree of telium production on them. Over a period of 4 years these suscepts have been carefully inoculated each year with aeciospore inoculum and have been exposed naturally to urediospore inoculum produced on heavily infected European black currants growing in the greenhouse in which the tests were carried out. As a preliminary report on the tests, it can be stated that infection was meagre, *e.g.*, on the *most susceptible* suscept only a very few telia formed, and after their formation these were observed to occur on tissue that became necrotic.

## Ribes rubrum Group

A second group according to the Thayer classification (13, p. 364) shows predominantly the morphological characters of *Ribes rubrum*. This group of varieties, as in the case of the *petraeum* group, is a small one, and is characterized by plants starting new growth late in the spring and holding their foliage late in the autumn. Varieties belonging to the *rubrum* group show considerable variation. They include the variety Victoria which may be regarded as typical of the group, the under side of the leaves being markedly pubescent.

Forms of *Ribes rubrum* that have been investigated (5, 12) produced scanty blister-rust infection or were highly resistant. The varieties studied included Victoria, Raby Castle, Gloire des Sablons, Dilnot Red, Scotch, and London Market (syn. London, London Red, Short Bunched Red), the last-named being very popular in some currant-growing sections—notably, southern Michigan (13).

Spaulding (12, Table 1) reported inoculation tests on at least 12 *rubrum* varieties. On all of these only a *slight* infection was recorded. The variety London was investigated more thoroughly than the others. Twenty-two tests were made; these indicated the variety to be highly resistant (nearly immune). Tests on *rubrum* forms were not qualified by stating the degree of telium production.

At a later date the writer (5) made greenhouse studies of 4 *rubrum* varieties (Victoria, Raby Castle, Scotch, London Market) recorded by Spaulding (12). The stock of Victoria and London Market was authenticated and received from Thayer (13). The writer likewise found that a slight production of fruiting bodies (uredia) occurred under optimum conditions for infection. Rust fruiting bodies did not form on Victoria and there is the possibility that this particular form may have been immune from blister rust.

At the Central Experimental Farm, Ottawa, the variety London Red, has been grown for 6 years, 1935 to 1940, in the blister-rust-test plots alluded to above. Only slight blister-rust infection was observed, although generally speaking the infection on nearby European black currants was considerable or very heavy during this period. London Red was free from infection in 1935, 1938, and 1939. As in the case of results reported by Spaulding (12), those from Canada were not qualified by stating the degree of telium production, which, from the standpoint of pine infection, is the most important stage.

Although *Ribes rubrum* has been reported susceptible to blister rust (12, 15), there are indications of rust resistance among its forms, *e.g.*, Tubeuf (15) cited the variety *glabellum* Trautv. and Mey. as immune from blister rust after 5 years' field testing. This form from northwestern Europe is late in blooming and also holds its foliage late. Spaulding (12, p. 52) too reported resistant varieties and described infections on "*R. rubrum* vars. *petrowalskyanum, pubescens,* and *siberica:* very scant sori, located beside large veins of leaf." Inasmuch as the habitat of the species includes a geographical range extending in northern Europe from Scotland, Scandinavia, Poland, and Finland to northern Russia, and from Siberia to eastern Manchuria, it would appear likely that the species would include rust-resistant forms native to the probable original home (Siberia) of white-pine blister rust.

Tubeuf (14, p. 300) rated *Ribes rubrum* growing in the wild state in Germany as producing a reduced degree of infection as compared with that on *R. nigrum* and *R. aureum* Pursh. Schellenberg (9) likewise reported the species growing in Switzerland to be exceedingly resistant to blister rust. Moreover, Spaulding,[5] in unpublished field notes made in Switzerland in August, 1922, while studying blister-rust conditions in Europe, recorded observations on *R. rubrum* made in company with Schellenberg at Zollikon, a few miles from Zurich. In a garden within 150 feet of badly diseased *Pinus strobus* L. planted 30 years earlier, these investigators found both the European black and wild red currants growing in association with other ribes. *R. nigrum* was very heavily infected, whereas only a single infection spot could be found on one leaf of *rubrum.* Spaulding recorded this comment: "The search on *R. rubrum* was careful and thorough as Schellenberg and I were arguing about infection of this species at the time." As

[5] Statement communicated through the courtesy of Dr. Perley Spaulding, Division of Forest Pathology, New Haven, Conn.

in the case of the rust-resistant *petraeum* varieties, it would be extremely valuable to know the exact form or forms of *rubrum* from which rust-resistant varieties of this latter group originated.

### Ribes sativum and R. sativum var. macrocarpum Groups

The remaining two red currant groups in Thayer's classification (13, pp. 363–4) in which the characters of *Ribes sativum* and *R. sativum* var. *macrocarpum*, respectively, predominate are large groups. The former included in the early 1920's almost all the varieties recommended for planting in this country. Concerning *R. sativum* (*vulgare*), Thayer (13, p. 317) had the following comment: "This species, which, by the way, has been badly confused in the past with *R. rubrum*, has for its habitat the dense woods, river banks and mountains of France from the Pyrenees to Brittany, Great Britain, Belgium, and possibly other countries of northern and western Europe. The true *rubrum*, on the other hand, is a native of lands much farther north. *R. vulgare* is readily recognized by the blossoms which are uncolored and which show a pentagonal raised ring or pad on the surface of the ovary. . . . *Vulgare* shows but little variation in the wild, and Janczewski gives but one botanical variety, *macrocarpum*. The origin of this variety is shrouded in mystery." The *sativum* group resembles the *macrocarpum* in earliness of leafing, blooming, and ripening, and also in an *early defoliation* (13, p. 373). The American Red Dutch, reported by the writer (6) as being rust-susceptible, according to Thayer (13, p. 373) is probably pure *sativum*.

The *Ribes sativum* groups include those varieties of the cultivated red currants now recognized as most susceptible to blister rust. A large percentage of the varieties reported by Spaulding (12) and the writer (5) belong to these groups and particularly to the *macrocarpum* section. Spaulding (12, Table 1), recording blister-rust infection, unqualified as to degree of uredium and telium production, and the writer (6, Fig. 2), reporting uredium production on varieties belonging to these two groups, indicated, as previously stated by the writer (5, p. 116), that cultivated reds can be considered as only a fair host for *Cronartium ribicola*, for fewer fruiting bodies are produced on them than on most Ribes, even when infected under the most favorable conditions. In estimating degree of infection, the writer compared uredium production on red currants to uredium production under favorable conditions on completely infected leaves of the very destructive European black currant, which was taken as a standard.

In addition, the writer (5, p. 107) commented upon the limited period of receptivity of red currant leaves to blister-rust infection, and Spaulding (12, pp. 52 and 54) described a common necrosis of leaf tissue resulting from infection. His plate V, fig. 4, shows infection on a variety originating from *Ribes sativum* var. *macrocarpum*. As might be expected, this necrosis, together with lesions resulting from other fungus and insect attacks,

very probably augments the natural early defoliation characteristic of the *sativum* and hybrid groups (3; 13, p. 373). In recent publications Snell (10, 11), in connection with very valuable studies of sporidia of *Cronartium ribicola* in relation to infection of white pine, has contributed additional data on necrosis of blister-rust infection spots on red-currant leaves in New York State, and also on defoliation of these plants during the early summer.

Telium production on infected leaves of red currants derived from *Ribes sativum,* has not received all the attention it should, particularly in the Northeast. Additional data are needed not only on the volume of telium and sporidium production on the most susceptible reds including those that have "escaped" to the wild, but also on the viability of telia on *sativum,* especially those on areas of infected leaf tissue which finally die. Further studies also should be made on the season of defoliation of infected red currants in the field and its effect upon white-pine infection.

## SUMMARY

Heretofore, in American blister-rust literature, all cultivated red-currant varieties have been lumped for convenience under *Ribes sativum* (Rchb.) Syme (*R. vulgare* Lam.), the most rust-susceptible species of this class of horticultural plants. In the light of our present knowledge of the taxonomical-pathological relationships of certain varieties that belong to other ribes species and that are highly resistant to or immune from *Cronartium ribicola* Fisch., such a treatment must now be considered obsolete.

According to Paul Thayer, whose classification is followed in the present paper, the cultivated reds originated from at least 3 European species—*Ribes petraeum* Wulf., *R. rubrum* L., *R. sativum,* and *R. sativum* var. *macrocarpum* Bailey. Blister-rust relations of cultivated red currants belonging to these groups are discussed.

The *Ribes petraeum* group is small and includes forms whose foliage, blossoms, and fruit appear late, the leaves hanging on the bushes until winter. Varieties belonging to *petraeum* are highly resistant to or immune from blister rust. In the literature the *petraeum* forms, Rivers Late Red, Long Bunch Holland (syn. Holland, Franco-German), Red Dutch (Holländische Rote), and Viking (syn. Rød Hollandsk Druerips), have been reported to be immune and Prince Albert to be highly resistant to blister rust.

Greenhouse inoculation tests on Red Dutch (Holländische Rote), that demonstrated the variety to be immune, are reported in this paper. These results corroborate field tests and observations by the late Professor Tubeuf in Germany.

The morphological characters of Red Dutch (Holländische Rote) and Viking are very similar. During the growing season both immune varieties are distinguishable from the common rust-susceptible garden varieties belonging to *Ribes sativum.*

Although the blister-rust relations of second generation Red Dutch seedlings have not been studied, those of Viking, previously published, showed the latter to breed nearly true for rust immunity. Selections of rust-immune Viking seedlings, some producing red and others white fruit, are reported in this paper. There is the possibility that some of these selections will be homozygous for rust immunity.

A preliminary report is made on telium production on the scant number of suscepts discovered during an earlier study of a large population of second-generation Viking seedlings. Over a period of 4 years (1937 to 1940) meager rust infection was obtained in greenhouse tests, *e.g.*, on leaves of the most susceptible seedling only a very few telia formed and these occurred on areas of leaf tissue that finally died.

The *Ribes rubrum* group likewise is a small one. It includes forms whose leaves are markedly pubescent on the under side. As in the case of the *petraeum* group, the leaves of the *rubrum* group are retained until late in the year. Forms belonging to *rubrum* show a very much reduced degree of infection or are highly resistant. The commercial variety, London Market, has been investigated most extensively and forms of it are reported to be nearly immune.

The *Ribes sativum* and *R. sativum* var. *macrocarpum* groups included in the early 1920's almost all the varieties recommended for planting in this country. The two groups are characterized by earliness of leafing, blooming, and ripening, and by an early defoliation. Most of the varieties that have been tested for blister rust belong to these two groups, which include forms now recognized as being most susceptible for red garden currants. Comparison is made between them and varieties derived from *petraeum* and *rubrum* with regard to their reaction to blister rust. It is pointed out that rust infection, together with lesions resulting from other fungus and insect attacks, very probably augment natural early defoliation of *sativum* varieties.

Despite a considerable amount of published work concerning the blister-rust relations of cultivated species of red currants, there are numerous questions pertaining to varieties belonging to the *Ribes sativum* group that need further investigation, *e.g.*, additional data on the volume of telium and sporidium production, the viability of telia, especially those on dead, infected leaf tissue, and field studies of the season of defoliation and its effect on white-pine infection.

DIVISION OF FOREST PATHOLOGY, BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE, COOPERATING WITH
THE OSBORN BOTANICAL LABORATORY,
YALE UNIVERSITY, NEW HAVEN, CONNECTICUT.

LITERATURE CITED

1. ANDERSON, O. C. A cytological study of resistance of Viking currant to infection by *Cronartium ribicola*. Phytopath. 29: 26–40. 1939.
2. BERGER, A. A taxonomic review of currants and gooseberries. New York State Agr. Exp. Stat. Tech. Bull. 109. 118 pp. 1924.

3. EWERT, R. Das Auftreten von *Cronartium ribicolum* auf verschiedenen Ribes-Arten in den Anlagen des Kgl. Pom. Inst. zu Proskau. Zeitschr. Pflanzenkr. 13 (2): 92–93. 1903.
4. HAHN, G. G. Preliminary report on a variety of red currant resistant to Weymouth pine rust. Trans. Bot. Soc. Edinb. 30: 137–146. 1929.
5. ————. A physiological method of distinguishing *Cronartium ribicola* and *C. occidentale* in the uredinial stages. Jour. Agr. Res. [U.S.] 40: 105–120. 1930.
6. ————. Immunity of Viking, a Norwegian red currant, to *Cronartium ribicola* and *C. occidentale* under greenhouse conditions. U. S. Dept. Agr. Circ. 330: 16 pp. 1935.
7. ————. Immunity of Viking red currant from white pine blister rust under field conditions. Phytopath. 26: 860–875. 1936.
8. ————. Blister rust susceptibility studies of naturally pollinated seedlings of the immune Viking currant. Jour. For. 36: 737–747. 1938.
9. SCHELLENBERG, H. C. Die Empfänglichkeit der Ribesarten für den Rost der Weymouthkiefer. Schweiz. Zeitschr. f. Forstw. 74: 25–50. 1923.
10. SNELL, W. H. Blister rust studies of three patches of red currants in New York. Phytopath. 31: 732–740. 1941.
11. ————. The production of sporidia of *Cronartium ribicola* on cultivated red currants in relation to infection of white pine. Am. Jour. Bot. 29: 506–513. 1942.
12. SPAULDING, P. Investigations of the white-pine blister rust. U. S. Dept. Agr. Bull. 957. 100 pp. 1922.
13. THAYER, P. The red and white currants. Their history, varieties, and classification. Ohio Agr. Exp. Stat. Bull. 371: 309–394. 1923.
14. TUBEUF, C. F. VON. Über das Verhältnis der Kiefernand-Peridermien zu Cronartium. III. Wirtpflanzen und ihre Disposition. Naturw. Zeitschr. Forst.-u. Landw. 15 (7/9): 296–302. 1917.
15. ————. Studien über Symbiose und Disposition sowie über Vererbung pathologischer Eigenschaften unserer Holzpflanzen. IV. Disposition der fünfnadeligen Pinus-Arten einerseits und der verschiedenen Ribes-Gattungen, Arten, Bastarde, und Gartenformen andererseits für den Befall von *Cronartium ribicola*. Zeitschr. Pflanzenkr. 43: [433]–471. 1933.
16. ————. Ausführung der organisierten praktischen Bekämpfung des Blasenrostes fünfnadeliger Kiefern. Zeitschr. Pflanzenkr. 45: 297–301. 1935.
17. ————. Verlauf und Erfolg der Erforschung der Blasenrostkrankheit der Strobe. Zeitschr. Pflanzenkr. 46: 49–103, 113–171. 1936.
18. WOOD, J. I., N. E. STEVENS, and D. REDDICK. Report of the Committee on Technical Words. Phytopath. 30: 361–368. 1940.

# OBSERVATIONS ON CERCOSPORA LEAF SPOT OF TOBACCO AND THE QUESTION OF VARIETAL RESISTANCE[1]

RUTH A. MCLEAN

(Accepted for publication July 25, 1942)

An outbreak of leaf spot caused by *Cercospora nicotianae* E. and E. on flue-cured tobacco occurred in fields at the Tobacco Experiment Station, Oxford, North Carolina, in 1941. Although this disease is present each year in North Carolina, it is usually of little importance. The early appearance and prevalence of spots in 1941 attracted attention, and, consequently, certain observations were made.

Cercospora leaf spot of tobacco has been reported to cause considerable damage to the crop in Queensland, Rhodesia, Sumatra, Malaya, and Nyasaland (14, 5, 23, 11, 22, 9). In Rhodesia (5) the yearly loss ascribed to it in 1939, was £100,000. The severity of the disease is conditioned by the prevailing weather and, accordingly, varies from year to year in a given location and in different localities.

## MATERIALS AND METHODS

The observations involved 2 to 4 rows each of 30 varieties of flue-cured tobacco planted for the production of seeds. About one-third of the plants of each variety had not been topped, and the seed heads had been bagged about 2 weeks before the first observations were made. Counts of all spots on all leaves of 10 plants of each variety were recorded for each leaf according to its position on the stalk. No attempt was made to determine the effect, if any, of topping on the susceptibility of plants to spotting. Identification of the pathogen was attempted by direct microscopic examination and study of cultures.

## OBSERVATIONS

Many minute circular to angular necrotic areas, 0.5 mm.–5.0 mm. in diameter, in which hyphae were found, but on which conidia were absent, occurred on all plants under observation. Hopkins (6) of Rhodesia reported similar spots in 1929. Again, in 1933 (7), he reported that Cercospora may produce symptoms almost typical of the brown spot caused by *Alternaria longipes* (E. and E.) Mason. He ascribed the abnormal appearance of lesions to a prolonged, cold, wet period at the beginning of the season followed by a severe drought.

The small lesions observed at the Oxford Experiment Station, when surface-disinfected and cultured, produced sterile mycelium. Potato-dextrose agar, as reported by Nagel (16), was used without success in an effort to secure sporulation. The fungus grew luxuriantly but produced only sterile hyphae on meat-extract agar containing 2 per cent dextrose. It did

[1] A cooperative investigation by the Departments of Botany and Chemistry, Duke University. The writer is indebted to Dr. F. A. Wolf and Dr. P. M. Gross for their assistance in the preparation of this paper.

not fruit on bits of infected tobacco leaf tissue that had been placed in humid chambers for 24–48 hours. Diachun (2) has reported that *Cercospora nicotianae* sporulates readily on tobacco-leaf-decoction agar when cultures are held for 48 hours in the dark at 27° C.

The small necrotic spots, first noted on July 24, increased little in size during the period July 24 to August 20, and the pathogen did not sporulate. The lesions observed would not have been recognized macroscopically during this period on the basis of the classic descriptions of mature Cercospora spots. However, 3 days of favorable weather, commencing August 19 with a rain during the night and continuing through August 22 (Table 1), in-

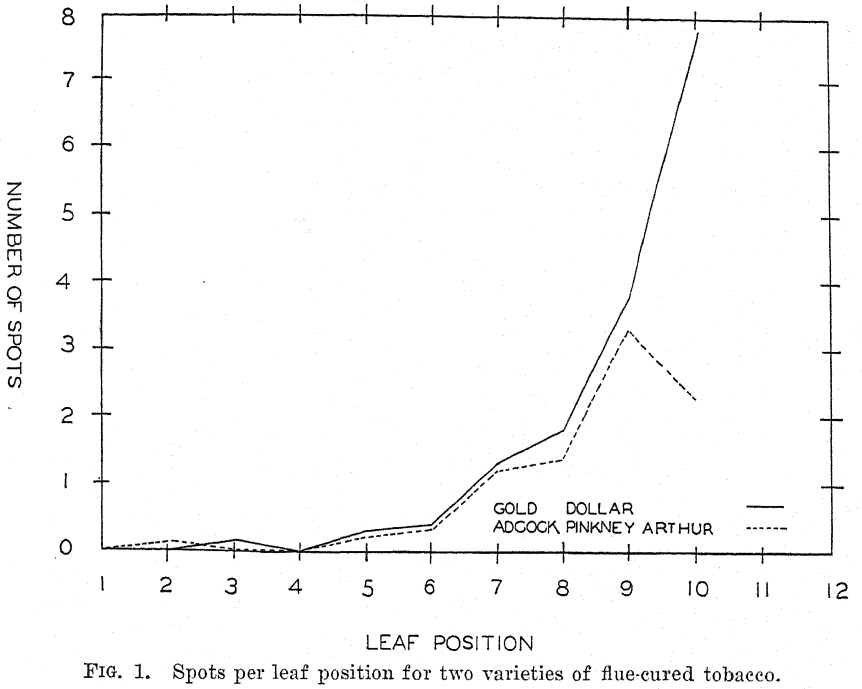



FIG. 1. Spots per leaf position for two varieties of flue-cured tobacco.

duced abundant sporulation on August 22, on more than 50 per cent of the lesions. Weather conditions during these 3 days differed from those of any 3-day period of the interval July 24 to August 19, in the number of hours the atmosphere was saturated, the amount of cloudiness, and in maximum temperatures that were less than 90° F. The interrelation of these factors on the growth and sporulation of *Cercospora nicotianae* is unknown. Mandelson (13) has determined that on culture media in the laboratory 45.5° and 93° F. are minimum and maximum temperatures respectively for growth. It may be assumed that the pathogen in the lesions which did not show sporulation on August 22 did not survive the extremely dry, hot weather of the preceding month.

Abundant sporulation by Cercospora on certain lesions and by Alternaria on others, figure 2, made possible a rapid and accurate differentiation

of spots in the field. The spots counted on July 24 proved, with few exceptions, to have been caused by Cercospora. Three observations, as listed below, are shown by the counts for two varieties, Gold Dollar and Adcock

FIG. 2. Tobacco leaf showing Cercospora spots left of midvein, upper left, and right of midvein, lower left. Cercospora spots are dull gray in contrast to the darker spots with white centers and concentric circles from which pure cultures of *Alternaria longipes* were obtained.

Pinkney Arthur (Fig. 1). The former is representative of varieties showing the highest count at the level of the tenth leaf from the top of the plant and the latter, of those showing the lowest count. Similar counts also were made in other plats on the farm.

1. Several varieties were almost spot-free for the 10th leaf from the top of the plant in contrast with certain other varieties.

2. In the case of all 30 varieties, the upper 5 leaves showed definite spotting but markedly less than the lower leaves.

3. A progressively higher count per leaf, the lower its position on the stalk, was noted in all varieties.

TABLE 1.—*Meteorological data in relation to sporulation of Cercospora nicotianae*

| Date | Precipitation[a] | Temperature | | Saturation No. hours per 24 hours | | Character of day | Sporulation of fungus |
|---|---|---|---|---|---|---|---|
| | | Min. | Max. | Hours | Period | | |
| | *In.* | | | | | | |
| July 24 | 0.32 | 61 | 88 | 10 | 8:00 p.m.– 6:00 a.m. | Clear | 0 |
| 25 | 0.00 | 61 | 92 | 7 | 11:00 p.m.– 6:00 a.m. | | 0 |
| 26 | 0.00 | 61 | 93 | 3 | 3:00 a.m.– 6:00 a.m. | Clear | 0 |
| 27 | 0.00 | 74 | 95 | 8 | 9:00 p.m.– 5:00 a.m. | | 0 |
| 28 | 0.00 | 72 | 95 | 3 | 4:00 a.m.– 7:00 a.m. | Clear | 0 |
| 29 | 0.00 | 73 | 97 | 12 | 8:00 p.m.– 8:00 a.m. | Clear | 0 |
| 30 | 0.38 | 71 | 95 | 6 | 2:00 a.m.– 8:00 a.m. | Clear | 0 |
| 31 | 0.15 | 74 | 95 | 12 | 8:00 p.m.– 8:00 a.m. | | 0 |
| Aug. 1 | 0.00 | 72 | 96 | 4 | 4:00 a.m.– 8:00 a.m. | Clear | 0 |
| 2 | 0.00 | 73 | 94 | 4 | 12:00 p.m.– 2:00 a.m. | | |
| | | | | | 4:00 a.m.– 6:00 a.m. | Clear | 0 |
| 3 | 0.00 | 70 | 92 | 5 | 3:00 a.m.– 8:00 a.m. | | 0 |
| 4 | 0.00 | 69 | 91 | 13 | 8:00 p.m.– 9:00 a.m. | Clear | 0 |
| 5 | 0.73 | 67 | 92 | 6 | 11:00 p.m.– 8:00 a.m. | | 0 |
| 6 | 0.00 | 68 | 91 | 0 | | | 0 |
| 7 | 0.00 | 62 | 92 | 0 | | Cloudy | 0 |
| 8 | 0.00 | 66 | 97 | 0 | | Clear | 0 |
| 9 | 0.00 | 68 | 99 | 0 | | Clear | 0 |
| 10 | 0.00 | 66 | 98 | | | | 0 |
| 11 | 0.00 | 65 | 95 | 6 | 12:00 p.m.– 6:00 a.m. | Clear | 0 |
| 12 | 0.80 | 70 | 95 | 0 | | Clear | 0 |
| 13 | 0.10 | 61 | 77 | 4 | 4:00 a.m.– 8:00 a.m. | | 0 |
| 14 | 0.00 | 49 | 89 | 5 | 3:00 a.m.– 8:00 a.m. | | 0 |
| 15 | 0.00 | 59 | 89 | 3 | 5:00 a.m.– 8:00 a.m. | Clear | 0 |
| 16 | 0.00 | 68 | 93 | 0 | | | 0 |
| 17 | 0.00 | 62 | 86 | 0 | | | 0 |
| 18 | 0.00 | 57 | 80 | 0 | | Decidedly cloudy entire day | 0 |
| 19 | 0.00 | 65 | 92 | 6 | 1:00 a.m.– 8:00 a.m. | Clear | 0 |
| 20 | 0.81 | 67 | 84 | 24 | 8:00 a.m.– 8:00 a.m. | Cloudy, gentle rain all day | 0 |
| 21 | 0.00 | 66 | 86 | 16 | 8:00 a.m.–12:00 a.m. | | |
| | | | | | 8:00 p.m.– 8:00 a.m. | Cloudy | 0 |
| 22 | 0.00 | 68 | 88 | 2 | 8:00 a.m.–10:00 a.m. | Cloudy, heavy fog | +Heavy |

[a] For 24 hours ending at sunset of date indicated.

METEOROLOGICAL DATA

Data are given in table 1 for the period from July 24, when the counts were begun, until August 22, when identity of most of the lesions was definite because of sporulation of the pathogen. Temperatures recorded were shade temperatures. A continuous record of the relative humidity and temperature was made by a Friez hygrothermograph placed 2 feet from the ground in a tobacco field on the Oxford experiment station farm.

## DISCUSSION

### General

In considering the observations recorded, it is interesting to note that the maximum number of spots occurring on any leaf was 8 (Fig. 1), whereas, in parts of Australia (14), in Ceylon (18), and perhaps in other places where conditions are favorable for the development of the disease, 100 spots or more per leaf are encountered in flue-cured tobacco. During some seasons in North Carolina, where there are decided variations in the amount of rainfall and the number of cloudy days, many more spots per leaf occur than the maximum recorded for 1941.

In attempting to draw conclusions from the results listed, the factors that modify infection, such as light, moisture, temperature, prevalence of inoculum and susceptibility of leaves must be considered.

### Sources and Distribution of Inoculum

Reports dealing with sources of inoculum of cercospora leaf spot of tobacco show that these sources differ according to varying locations and cultural practices (17, 21, 25, 8, and 3).

The presence of species of wild tobacco, susceptible to cercospora infection in the vicinity of tobacco fields, is listed as a source of inoculum (3). It is suggested that until more extensive cross-inoculation tests are made, the possibility of host plants other than those listed by Hill (3) serving as sources of inoculum, should not be overlooked. However, regarding airborne spores Park (17) has shown that cercospora spores were not transmitted, in a viable condition, as much as 100 yards by air currents.

Inasmuch as cercospora is known to be seed-borne (25), it is possible that spores that caused the spots counted on July 24, were produced on lesions of the lower leaves of the young plants in the field or could have been present on the seedlings at the time of transplanting. In such case, it is probable that more spores would have reached the lower than the upper leaves during the period favorable to infection. The same would have been true had the soil in the field, a source considered by Park (17) of primary importance in Ceylon, been responsible for the outbreak.

### Increased Susceptibility of Lower Leaves and Varietal Differences in Degrees of Spotting

Experimental work to determine whether there was an equal distribution of inoculum from the uppermost to the lowest leaves on the stalks was

not done, nor was inoculum artificially applied to insure its equal distribution on leaves at all levels on plants under observation. Observational evidence, however, permits the conclusion that the lower leaves were more susceptible to invasion by the fungus than the upper leaves.

The counts (Fig. 1) show unquestionably that a greater number of spots were present, on a given date, on the lower than on the upper leaves. It is the generally expressed opinion, though not supported by experimental evidence, that the lower a tobacco leaf is on the stalk, and consequently the riper, the more susceptible it is to cercospora infection (6, 4, 13, 14, 7, 24). This opinion also accords with observations by Shear (20) in regard to physiological spotting of flue-cured tobacco leaves and those of Anderson (1) in regard to the John Williams Broadleaf spot of shade-grown cigar tobacco. Differing observations, however, in respect to cercospora spotting are expressed by Hornby (10) and Schweizer (19).

An unpublished report on inoculation studies of *Alternaria longipes* (12) contains evidence that the fungus involved is capable of invading leaves or sections of leaves that have reached a certain degree of maturity, but that under precisely the same conditions otherwise cannot establish itself on younger leaves or portions of a leaf.

Mandelson (13) has presented experimental evidence that more rapid destruction of leaf tissue by *Cercospora nicotianae* takes place in mature than in less mature leaves. This accords with the statement by Mohr (15) in Sumatra, that plants of *Nicotiana triplex,* which, in contrast with other Deli varieties and foreign tobaccos, showed some degree of resistance to cercospora spotting, had no fewer spots than less resistant varieties, but that the spots were smaller.

It is known that leaves at the same stalk level on different plants of one variety, grown under presumably comparable conditions, ripen on different dates. Likewise, certain varieties ripen earlier than others. Definite criteria for judging ripeness have not been established. Consequently, data other than stalk position regarding the condition of leaves at the time counts were made, are not available for correlation with counts of spots.

The difference for the 2 varieties having the most widely divergent counts for the tenth leaf from the top of the plant is shown graphically (Fig. 1). A varietal difference in susceptibility seemed on first thought a possible explanation. Statistical analyses showed that Gold Dollar, the variety having the greatest average count for the tenth leaf from the top, differed significantly in this respect from Special 400, Adcock Pinkney Arthur, Mammoth Gold, Coker's 1940 Selection and Jamaica Huggins. Due to the rapid ripening and harvest of leaves in the field under observation, counts of spots on leaves at stalk levels lower than the tenth were not obtained in sufficient numbers to justify conclusions in regard to them. Counts at any leaf position for remaining 25 varieties did not differ significantly from those of Gold Dollar.

An uneven distribution of inoculum could have accounted for the differences in counts obtained for different varieties. However, in the case of

the field under observation, presumptive evidence that an even distribution of inoculum occurred over the entire variety field is presented in the counts obtained for variety 401 in an adjoining field and in the field in question. Counts of spots on 100 leaves, at the level of the tenth from the top of the plant, variety 401, widely distributed in a 2-acre field, did not differ significantly from counts for leaves at the same stalk level, variety 401, at a distance of 1000 ft., in the variety field. There is admittedly a remote chance that a gradient could have existed across the field, in the concentration of inoculum. This possibly could have existed by coincidence, depending upon the source of inoculum, for the variety field and not for the field compared to it. Strong evidence that this did not occur and that an even distribution of inoculum, whatever its source and the means of distribution, prevailed over the entire variety field for any given stalk position, is found in the examination of counts for Gold Dollar growing in two rows along the edge of the field and in two rows two-thirds of the distance across the field. Counts for any leaf position of this variety were practically the same for both locations in the field.

However, the differences between varieties noted for the tenth leaf from the top of the stalk were not found in the case of leaves at higher levels, for example, leaves 7, 8, and 9 from the top of the plant. One exception to this was in the case of the eighth leaf from the top of the plant in Jamaica Huggins. This makes it appear unlikely that varietal resistance exists among these different types because presumably it should operate not only for the lowest leaf on the stalk, but also for the next higher and for leaves at all levels. On the other hand, the fact that counts (Fig. 1) at all stalk levels downward through the ninth leaf were essentially the same for the varieties that differed widely in the count for the tenth leaf from the top of the plant, is presented as an indication that the condition of the leaf as regards ripeness determines its degree of susceptibility to cercospora spotting.

The present data and observations emphasize the necessity for careful scrutiny of the evidence for the existence of differences in varietal resistance. The evidence should be examined from the point of view of even distribution of inoculum, equally favorable atmospheric conditions for infection at any given stalk level and equal ripeness of leaves compared. In regard to distribution of inoculum and equally favorable conditions for infection, leaves from all the plants for any given stalk position were probably comparable at any given stalk level. However, the remote possibility that the tenth leaf from the top of the plant in Gold Dollar was, on the average, lower on the stalk than was the tenth leaf in the varieties from which it differed significantly, should be considered in an attempt to explain differences in numbers of spots. If this had been true, and the soil an important source of inoculum, a higher count would have been expected for the lowest leaves. In such case the amount of inoculum reaching the ascending leaves would have been proportionately greater in this variety than in the others. Dif-

ferences in amounts of spotting by spread of inoculum upward from the lowest leaves by means of air currents, insects, and spattering rain might be accounted for by differences in infection of seedlings when removed from the seed bed. The influence of these factors on the infections observed is unknown.

The leaves of the different varieties that were compared, though at the same stalk levels, were not known to be of equal ripeness. It is likely that varietal differences known to condition the time necessary for maturing of leaves account for the differences observed in susceptibility to spotting. It is not possible to claim the existence of definite evidence for the presence of varietal resistance independent of maturity of leaves, on the basis of the available data. This is in agreement with the decision of van der Weij (24) who made observations that he thought possibly indicated varietal resistance. In order to secure definite evidence of differences in varietal susceptibility to cercospora leaf spot, it would be necessary to determine by preliminary inoculation tests, the chronological age at which leaves of different varieties developed a maximum number of spots under standardized conditions. Then, if standardized conditions could be maintained throughout the tests, counts of spots, made for leaves at certain stalk positions for each variety on the date when leaves in these positions were known to be most susceptible, would be significant in comparing varieties for resistance to spotting.

## SUMMARY

An outbreak of leaf spot caused by *Cercospora nicotianae* on flue-cured tobacco occurred in the fields at the Tobacco Experiment Station, Oxford, North Carolina, in 1941. Most of the lesions remained atypical in appearance during the greater part of an unusually dry, hot growing season and would not have been recognized macroscopically on the basis of the classic descriptions of mature frog-eye spots. Cultural studies were made to determine identity of the fungus. Identity of the causal fungus was definitely confirmed when a prolonged rainy period during the latter part of the season induced abundant sporulation of the fungus in a large percentage of the lesions under observation.

Several varieties were almost free of spots for the tenth leaf from the top of the plant in contrast with certain other varieties. Definite evidence of differences in varietal susceptibility cannot be claimed as the leaves compared, although of the same chronological age, were not known to be of equal ripeness.

Evidence is presented to show that apparent differences in susceptibility of two varieties were probably due to the earlier ripening of the lower leaves of one variety than of those of the other, rather than to a specific resistance of one variety to the pathogen.

In all varieties the upper 5 leaves showed definite spotting but markedly less than the lower leaves.

In all varieties a progressively higher count per leaf, the lower its position on the stalk, was noted.

DUKE UNIVERSITY,
DURHAM, NORTH CAROLINA.

LITERATURE CITED

1. ANDERSON, P. G., T. R. SWANBACK, and O. E. STREET. Leaf spots. Report of the Tobacco Sub-station at Windsor. Conn. Agr. Exp. Stat. Bull. 367, p. 128. 1934.
2. DIACHUN, STEPHEN, and W. D. VALLEAU. Conidial production in culture by *Cercospora nicotianae*. Phytopath. **31**: 97–98. 1941.
3. HILL, A. V. Cercospora leaf spot of tobacco in Queensland. Bull. Coun. Sci. Indus. Res. (Australia) **98**. 1936.
4. HOPKINS, J. C. F. Annual report of the Senior Plant Pathologist for the year ending Dec. 31, 1936. Rhodesia Agr. Jour. **33**: 413–421. 1936.
5. ————. Diseases of tobacco in Southern Rhodesia. (Supplement I, 1932 to 1938) Rhodesia Agr. Jour. **36**: (11) 45–65. 1939.
6. ————. Frog-eye disease of tobacco. Rhodesia Agr. Jour. **26**: (8) 817–822. 1929.
7. ————. Mycological notes. Seasonal notes on tobacco diseases. 6. An unusual type of frog-eye spotting. Rhodesia Agr. Jour. **30**: (6) 472–474. 1933.
8. ————. Mycological notes. Seasonal notes on tobacco diseases. 5. Evil effects of delayed "priming." Rhodesia Agr. Jour. **30**: (2) 120–123. 1933.
9. HORNBY, A. J. W. Annual report of the Department of Agr. Nyasaland, 41, 1932.
10. ————. Report of the Assistant Director and Agricultural Chemist, Ann. Rept. Dept. Agr. Nyasaland, **1932**: 36–47. 1933.
11. JOCHEMS, S. C. J. Spikkel in Deli tabak (Cercospora disease on Sumatra tobacco). Meded. Deli Proefst., Medan-Sumatra. Tweede Serie, No. 72.
12. MCLEAN, R. A. Alternaria in relation to brown spot of tobacco. (Unpublished manuscript.)
13. MANDELSON, L. F. Barn spot of tobacco. Preliminary investigations and flue-curing experiments. Queensland Agr. Jour. **41**: 132–147. 1934.
14. ————. Frog-eye leaf spot and barn spot of tobacco. Queensland Agr. Jour. **40**: (5) 401–408. 1933.
15. MOHR, J. C. VAN DER MEER. Verslag van het Deli Proefstation over het jaar, 1936. Meded. Deli Proefstat. **97** (2). 1937.
16. NAGEL, C. M., and S. M. DIETZ. The sporulation of five species of *Cercospora* in pure culture. Phytopath (Abstract) **22**: 20. 1932.
17. PARK, M., and M. FERNANDO. Some studies on tobacco diseases in Ceylon. I. Trop. Agr. **88**: (3) 153–168. 1937.
18. ———— and ————. Some studies on tobacco diseases in Ceylon. II. Field spraying against frog-eye (*Cercospora nicotianae* E. and E.). Ibid. **88**: (5) 266–282. 1937.
19. SCHWEIZER, J. Annual report on tobacco from July, 1937, to June, 1938. Meded. Besoeck. Proefstat. 62. 1938.
20. SHEAR, G. M. Factors affecting physiological breakdown of maturing tobacco. Va. Agr. Exp. Stat. Tech. Bull. 74, pp. 3–16. 1941.
21. SIMMONDS, J. H. Plant pathological investigations. Rep. Dept. Agr., Queensland. 82–84. 1936–37.
22. THOMPSON, A. Annual report of the mycologist for 1923. Malayan Agr. Jour. **12**: 246–251. 1924.
23. WEIJ, H. G. VAN DER. Report on the diseases and pests of Deli tobacco in the year 1937. A. Tobacco diseases. Meded. Deli-Proefst. Ser. 2, **98**: (2) 3–9. 1938.
24. ————. Resistentie—selectie tegen Spikkel. Verslag van het Deli Proefstation over het jaar, 1937. Meded. Deli Proefstation (**1937**): (2) 13–14. 1938.
25. WOLF, F. A. Tobacco diseases and decays. 454 pp. (Duke Univ. Press.) 1935.

# SOME RELATIONSHIPS BETWEEN POTATO YELLOW-DWARF VIRUS AND THE CLOVER LEAF HOPPER

L. M. Black

(Accepted for publication September 17, 1942)

## INTRODUCTION

It is the purpose of this note to make available hitherto unpublished miscellaneous information on the clover leaf hopper, *Aceratagallia sanguinolenta* Prov., and the potato yellow-dwarf virus, *Marmor vastans* H. var. *vulgare* Black. Data on the transmission of the virus by nymphs, the failure of the virus to affect insect mortality, and the incubation period of the virus in crimson clover, *Trifolium incarnatum* L., were accumulated incidentally during studies on genetic variation in the ability of the clover leaf hopper to transmit potato yellow-dwarf virus (3). In addition, the paper includes an account of experiments on the incubation period of the virus in the insect, the frequency of transmission of virus, and the failure of the virus to pass through the insect egg. These experiments were either performed too late for inclusion or were unsuitable for inclusion in earlier papers.

## TRANSMISSION BY NYMPHS

Transmission of potato yellow-dwarf virus by adults and nymphs of the clover leaf hopper has been observed in hundreds of cases in controlled experiments. In experiments on active and inactive races of the clover leaf hopper (3), the presence or absence of a cast skin was recorded whenever individual insects were transferred to fresh test plants. The instar or instars in which an insect had transmitted the virus was determined by counting these cast skins back from the last ecdysis. Failure to find a skin that actually had been cast would therefore result in recording an infection by an instar older than that in which the transmission took place. Thus, the small number of errors of this sort which may have been committed do not invalidate the conclusions drawn from the data. According to Watkins (6), the clover leaf hopper passes through 5 nymphal instars, and this number has been assumed for all insects in the present study, although in an experiment described below evidence for some variation in the number of instars was obtained. Altogether, 0 transmissions were recorded for the 1st instar, 1 for the 2nd, 17 for the 3rd, 61 for the 4th, and 227 for the 5th. It seems safe to conclude, at least, that transmission occurred in the 3rd, 4th, and 5th nymphal instars. There is a remote chance that the record of a single transmission by a nymph in the 2nd instar may be due to a contamination or a mistake in recording data. The data should not be construed as indicating that transmission does not occur in the 1st instar because in the experiments from which these data were derived the earlier the instar the fewer the insects tested.

FAILURE OF VIRUS TO AFFECT INSECT MORTALITY

It is interesting that not a single plant virus has been found to affect its insect vector in any way other than to render it infective. In an attempt to discover whether or not the potato yellow-dwarf virus had a deleterious influence on the clover leaf hopper, the mortality of the leaf hoppers in the genetic studies (3) was analyzed in relation to their infectivity. The odds for any difference not being due to chance alone were derived by the use of the 4-fold table. The chi-square values were translated into odds by using corresponding probability values in Fisher's (4) or Yule's (7) tables. To facilitate the analysis, neither insects dying as nymphs nor any of the hybrids between the active and inactive races were included. Insects dying on their first test plant also were not included, because they were considered to have been inadequately tested for infectivity. Analysis of the data on the

TABLE 1.—*Insect mortality in various population groups*

| Infectivity | Race | Total insects | Insects dying | Percentage of insects dying | Odds that difference not due to chance alone |
|---|---|---|---|---|---|
| Infective | Active | 562 | 81 | 14.4 | |
| | Inactive | 78 | 8 | 10.3 | |
| | Total | 640 | 89 | 13.9 | |
| Noninfective | Active | 163 | 32 | 19.6 | > 100 : 1[a] |
| | Inactive | 698 | 76 | 10.9 | |
| | Total | 861 | 108 | 12.5 | |
| Both | Active | 725 | 113 | 15.6 | 97 : 1[a] |
| | Inactive | 776 | 84 | 10.8 | |

[a] These were the only differences found to be significant.

rest of the insects reveals that out of 640 infective individuals 89, or 13.9 per cent, died, whereas out of 861 noninfective individuals 108, or 12.5 per cent, died. This difference is not significant (odds 1 : 1) ; that is, the chances were only 1 : 1 that the difference was not fortuitous. However, when the infective and noninfective insects in each race were considered separately (Table 1), the data revealed a possible tendency for a higher death rate among noninfective than among infective insects in the active race (odds 5 : 1). This difference may be fortuitous or, if there be such a tendency, it may be nothing more than a reflection of the probability that the death of some insects during the test period prevented them from shifting from the noninfective to the infective group. If the data are considered from the standpoint of race (Table 1), it is apparent that there is a significant difference between the two races. More deaths occurred in the active race, independently of whether the insects were infective or not. The higher death rate in the active race is attributed to the effects of inbreeding which fortuitously were more harmful in the active race than in the inactive one. When the data were reconsidered on the basis of sex, it was found that out of 731 males 103, or 14.1 per cent, died, and out of 770 females 94, or 12.2 per cent, died. This

difference is not significant (odds 2:1). The data reveal no evidence that the potato yellow-dwarf virus hastens the death of clover leaf hoppers transmitting it.

### INCUBATION PERIOD OF THE VIRUS IN CRIMSON CLOVER

During studies on the genetic variability of the clover leaf hopper in transmitting potato yellow-dwarf virus (3), the week in which each clover test plant developed symptoms was recorded. These data have been collected and arranged in table 2. The test plants upon which the 5th generation of insects were tested were omitted from the table, since weekly records were not made on them. Usually, insects were kept on test plants for 1 week; if the period exceeded 1 week the resulting data were also omitted

TABLE 2.—*Incubation period of potato yellow-dwarf virus in crimson clover*

| Experimental period | Insect generation tested | Number of plants developing symptoms in the | | | | |
|---|---|---|---|---|---|---|
| | | 1st week | 2nd week | 3rd week | 4th week | 5th week |
| | | after the inoculation period of 1 week | | | | |
| Feb. 3, 1937 to Apr. 28, 1937 | P | 1 | 36 | 15 | 7 | 0 |
| Apr. 28, 1937 to June 9, 1937 | $G_1$ | 0 | 22 | 11 | 4 | 0 |
| Sept. 1, 1937 to Nov. 10, 1937 | $G_2$ | 2 | 29 | 10 | 7 | 1 |
| Dec. 4, 1937 to Feb. 27, 1938 | $G_3$ | 3 | 159 | 164 | 70 | 21 |
| Mar. 18, 1938 to May 20, 1938 | $G_4$ | 19 | 96 | 43 | 16 | 10 |
| Oct. 3, 1938 to Nov. 30, 1938 | $G_6$ | 1 | 45 | 57 | 33 | 13 |
| Dec. 15, 1938 to Mar. 2, 1939 | $G_7$ | 2 | 61 | 79 | 53 | 29 |
| Apr. 27, 1939 to June 15, 1939 | $G_8$ | 1 | 17 | 33 | 16 | 8 |
| June 29, 1939 to Aug. 31, 1939 | $G_9$ | 3 | 80 | 122 | 42 | 18 |
| Total | | 32 | 545 | 534 | 248 | 100 |

from the table. The experimental period (Table 2) includes the period during which the plants were inoculated and the subsequent period during which they were observed in the greenhouse. Incubation periods were reckoned from the middle of the feeding period of 1 week. For example, if a plant developed symptoms during the following week, the fact was recorded in table 2 under the 1st week. This method of recording the data is believed to give a fairly accurate picture of the variation in the incubation period. However, it must be pointed out that no case of an incubation period as short as one week was observed, *i.e.*, no plant was observed to have symptoms of disease when the insect was removed from it at the end of the 1-week feeding period. It is apparent from the table that the incubation period is quite variable and that most plants develop the first symptoms of the disease in

the 2nd and 3rd weeks. It is also evident from the totals that some plants may be expected to develop symptoms after the 5th week but that this number will not be an important fraction of the plants inoculated.

### INCUBATION PERIOD IN THE INSECT

As the result of a preliminary experiment, the incubation period of potato yellow-dwarf virus in the clover leaf hopper was reported in an earlier paper as being about 9 days (1). Since that time additional experiments have been performed. The experiments are summarized in table 3. Altogether, 8 experimental and 1 control series of transfers were carried out. In all series the nonviruliferous insects were fed on potato plants (*Solanum tuberosum* L. var. Green Mountain) for 24 hours before beginning the series of daily transfers on fresh healthy test plants. During this preliminary 24 hours, the control series was fed on healthy plants, the others on the tips of stems in the acute stage of yellow dwarf. In series 2 the experiment was begun with adults, in all others with nymphs. Many of the nymphs changed to adults before the transfers were completed. In series 1, 2, and 3, young healthy potato plants were employed as test plants; in the other series, crimson clover plants were used. The clover test plants had about 4 trifoliate leaves when the insects were added. The number of insects surviving at each transfer and the presence or absence of yellow dwarf in the test plants are given in table 3. It is evident from the table that the potato yellow-dwarf virus had a variable incubation period in the clover leaf hopper, a minimum of 6 days (series 4) and a maximum of 10 days (series 6, 7) being detected. It seems probable, however, that if incubation periods had been determined for single insects much longer intervals would have been recorded for some individuals.

This seems the more likely if one considers data obtained by testing single insects in the genetic studies (3). In these studies there were 133, 65, 42, 42, and 8 insects that failed to transmit until the 2nd, 3rd, 4th, 5th, and 6th weeks, respectively, after completing their feeding period on diseased plants. It is believed that these delays probably represent long incubation periods because it seems probable that, in most cases, these insects did not transmit virus during the period they were reared on diseased plants. However, because the period the insects fed on diseased plants varied from about 2 to 4 weeks, it is impossible to be certain that these long periods represent incubation periods and not long lapses between transmissions. To make certain of such a long incubation period as the 6-week period indicated above would require the testing of single insects on a large scale after permitting them to pick up virus for one day only.

### FREQUENCY OF TRANSMISSION

To determine the frequency with which the clover leaf hopper transmits potato yellow-dwarf virus, 2 experiments were carried out in which each of several infective insects was transferred daily to a fresh healthy crimson

TABLE 3.—*Incubation period of potato yellow-dwarf virus in the clover leaf hopper*

| Test plant | Series | Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Potato | Experimental 1 | Number of insects | 127 | 127 | 124 | 121 | 121 | 121 | 121 | 119 | 119 | 110 | 108 | 108 | 105 | 96 | 81 | 63 | 61 | 50 | 44 | 37 | 27 | 24 | 21 | 21 | 21 | 21 |
| | | Plant reaction | − | − | − | − | − | − | − | − | − | + | * | − | * | * | * | * | * | + | * | * | − | − | + | + | + | − |
| | Experimental 2 | Number of insects | 100 | 98 | 95 | 95 | 91 | 85 | 83 | 81 | 80 | 77 | 73 | 65 | 64 | 61 | 60 | 57 | 55 | 53 | 48 | 47 | 43 | 42 | 39 | 37 | 36 | |
| | | Plant reaction | − | − | − | − | − | − | − | − | + | − | − | − | − | − | + | − | + | + | − | − | − | − | + | + | − | |
| | Experimental 3 | Number of insects | 128 | 112 | 111 | 103 | 100 | 100 | 98 | 98 | 98 | 98 | 98 | 98 | 96 | 93 | 92 | 83 | 80 | 73 | 69 | 62 | | | | | | |
| | | Plant reaction | − | − | − | − | − | − | − | + | + | + | + | + | + | + | + | + | + | + | − | + | | | | | | |
| Crimson clover | Experimental 4 | Number of insects | 81 | 74 | 70 | 66 | 63 | 60 | 60 | 59 | 59 | 56 | 55 | 54 | 54 | 54 | 54 | 54 | 54 | 54 | 51 | 51 | | | | | | |
| | | Plant reaction | − | − | − | − | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | + | | | | | | |
| | Experimental 5 | Number of insects | 100 | 94 | 85 | 78 | 72 | 69 | 64 | 59 | 57 | 57 | 55 | 55 | 52 | 50 | 48 | 48 | 48 | 47 | 47 | 47 | | | | | | |
| | | Plant reaction | * | * | − | − | − | − | − | − | + | + | + | − | − | − | − | − | + | − | − | + | | | | | | |
| | Experimental 6 | Number of insects | 100 | 96 | 93 | 89 | 79 | 78 | 65 | 60 | 60 | 59 | 59 | 58 | 57 | 57 | 57 | 54 | 53 | 53 | 53 | 53 | | | | | | |
| | | Plant reaction | − | − | − | − | − | − | − | − | − | − | + | + | − | + | + | + | + | + | + | + | | | | | | |
| | Experimental 7 | Number of insects | 100 | 99 | 97 | 97 | 90 | 73 | 64 | 60 | 60 | 59 | 57 | 56 | 56 | 56 | 56 | 53 | 51 | 51 | 51 | 50 | | | | | | |
| | | Plant reaction | − | − | − | − | − | − | − | − | − | − | + | + | + | + | − | − | + | + | + | + | | | | | | |
| | Experimental 8 | Number of insects | 100 | 95 | 95 | 93 | 91 | 91 | 86 | 86 | 83 | 83 | 79 | 78 | 78 | 77 | 75 | 75 | 73 | 73 | 72 | 72 | | | | | | |
| | | Plant reaction | − | − | − | − | − | − | − | − | − | + | − | − | + | − | − | + | + | + | − | + | | | | | | |
| | Control 1 | Number of insects | 95 | 88 | 86 | 83 | 75 | 73 | 63 | 59 | 59 | 58 | 58 | 57 | 55 | 51 | 51 | 49 | 49 | 48 | 48 | 48 | | | | | | |
| | | Plant reaction | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | | | | | | |

* = plant died due to causes other than yellow dwarf. + = plant developed yellow dwarf. − = plant remained healthy.

clover seedling. In both experiments the insects were members of the active race (3), the insects of the 1st and 2nd experiments coming from the 7th and 8th generations, respectively. In both cases the insects were hatched and reared on diseased crimson clover and began their series of daily transfers as nymphs. In the 2nd experiment data as to sex and ecdyses were recorded and 2 control plants to which no insects had been added were included each day. Plants were held for observation for at least 6 weeks after the insects were placed on them. The results are presented in table 4.

It is apparent from the table that there is great variation in frequency of transmission between different insects and within the same insect at different times. Insect 8, experiment 2, transmitted only on the 16th and 17th days that it was tested on healthy plants and not on any of the subsequent 27 days that it was tested. On the other hand, insect 12, experiment 1, infected 25 out of the 30 plants on which it was tested. It infected as many as 10 successive plants. It is apparent that the virus remains in the insect through ecdysis; other than that, there is no obvious relation between ecdysis and transmission. There are many periods of several days between transmissions. For example, insect 12, experiment 2, transmitted only on the 8th, 24th, and 40th days. Insect 11, experiment 2, transmitted on the 10th day and then failed to transmit until the 35th day, more than 3 weeks later. On searching the data connected with the genetic studies (3), it was found that there were many such lapses in transmission. Altogether, there were 149 cases of a lapse of 1 week, 36 cases of a lapse of 2 weeks, and 8 cases where the lapse lasted for 3 weeks. The presence of some weed seedlings growing at random throughout the cages may account in part for lapses of transmission in the genetic studies (3). This, however, could not be a factor in the experiments in which the insects were transferred daily, since the pots were always carefully weeded before the plant was caged and the insect added.

The experiment also provides evidence that active insects remain infective for at least 44 days under conditions where it is very improbable that they could acquire fresh virus from plants. In other preliminary experiments the insects remained infective for at least 52 days while feeding on rye. It has been reported earlier (2) that the leaf hoppers remain viruliferous while hibernating without access to plants for at least 167 days, that overwintering adult leaf hoppers collected in the field in the early spring were infective, and that virus could be demonstrated in the body of the insect in any season. It seems clear from these various observations that the potato yellow-dwarf virus is retained by the clover leaf hopper for long periods.

### FAILURE OF VIRUS TO PASS THROUGH THE INSECT EGG

Fukushi (5) has reported the only known case in which a plant virus passes from parent to offspring through the egg of the insect vector. To ascertain whether or not the potato yellow-dwarf virus passes from parent to young by way of the egg or sperm, an experiment was designed to test

TABLE 4.—*Frequency of transmission of potato yellow-dwarf virus by individual clover leaf hoppers*

| Experiment | Insect | Sex | Day 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | Undetermined | – | – | – | – | – | – | – | D | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 2 | | + | – | + | + | + | + | – | + | – | D | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 3 | | – | + | + | + | – | + | + | – | – | – | D | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 4 | | – | – | + | + | – | + | + | + | + | + | D | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 5 | | – | – | – | – | – | – | + | – | – | – | – | – | – | – | – | – | – | – | – | – | D | | | | | | | | | | | | | | | | | | | | | | | | |
| | 6 | | – | – | – | – | + | – | – | + | – | – | + | – | – | – | – | – | – | – | – | + | D | | | | | | | | | | | | | | | | | | | | | | | | |
| | 7 | | + | – | – | + | – | + | – | + | + | + | + | + | + | – | – | + | – | + | – | – | – | D | | | | | | | | | | | | | | | | | | | | | | |
| | 8 | | + | + | + | – | + | + | – | – | + | – | + | + | + | + | – | – | + | + | + | + | + | D | | | | | | | | | | | | | | | | | | | | | | |
| | 9 | | – | + | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | D | | | | | | | | | | | | | | | | | | |
| | 10 | | – | – | – | – | + | + | – | + | + | + | – | + | + | + | – | + | + | – | – | + | + | + | + | + | + | L | | | | | | | | | | | | | | | | | |
| | 11 | | + | + | + | + | – | + | – | + | + | + | + | + | + | + | + | + | – | + | + | + | + | – | – | + | – | + | + | + | – | | | | | | | | | | | | | | |
| | 12 | | – | + | + | + | + | + | + | + | + | + | – | – | + | + | + | + | – | + | + | + | + | + | + | + | + | + | – | + | + | | | | | | | | | | | | | | |
| 2 | 1 | ♀ | – | – | – | –c | – | + | – | D | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 2 | ♂ | – | + | – | – | –a | – | – | + | – | – | – | – | – | – | – | – | + | – | – | – | + | + | – | – | – | + | – | – | D | | | | | | | | | | | | | | |
| | 3 | ♀ | – | – | –a | + | – | – | + | – | – | + | – | + | – | – | + | – | – | – | – | – | – | + | – | – | – | – | – | – | – | – | + | – | – | – | + | – | – | + | – | – | + | + | D | |
| | 4 | ♀ | – | – | – | – | – | – | – | – | – | – | – | –a | – | – | – | – | – | – | – | – | – | – | – | + | – | – | – | – | + | – | – | – | – | – | – | + | – | – | – | – | – | + | – | + |
| | 5 | ♂ | + | + | – | –a | – | – | + | + | – | + | – | + | + | – | – | – | – | + | – | + | – | – | – | – | – | + | – | + | + | + | + | + | – | + | + | – | – | – | – | + | – | + | – |
| | 6 | ♀ | + | – | – | –a | – | – | – | + | – | + | – | + | – | – | – | – | – | + | – | – | – | – | – | – | + | + | – | – | – | – | + | – | – | + | – | – | + | + | – | – | – | – | – | – |
| | 7 | ♂ | – | – | – | – | –a | – | – | – | – | – | – | + | – | – | – | – | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | + | – | – | – | + | – | – | – | – | – |
| | 8 | ♂ | – | – | – | – | –a | – | – | – | – | – | – | – | – | – | – | + | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | 9 | ♀ | – | – | – | –a | – | – | – | – | – | – | + | – | – | – | – | + | – | – | – | + | – | – | – | – | – | – | – | – | – | – | – | – | – | + | – | – | + | – | + | – | – | – | – | – |
| | 10 | ♀ | – | – | – | – | – | – | – | – | – | –a | – | – | – | – | – | – | – | – | – | – | – | – | – | + | + | + | – | – | – | – | + | – | – | + | – | + | – | – | – | – | + | + | – |
| | 11 | ♀ | – | – | – | – | –a | – | – | – | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | + | – | – | – | + | + | + | – | + | – |
| | 12 | ♂ | – | – | –c | – | – | – | – | + | – | – | – | – | –a | – | – | – | – | – | – | – | – | – | – | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | + | – | – | – | – |
| | 13 | ♀ | – | – | – | – | –c | – | – | – | + | – | – | – | – | – | – | – | – | –a | – | – | – | – | – | + | + | – | + | + | + | – | – | + | – | – | – | – | – | – | – | – | – | – | – | – |
| Control | 1 | | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | 2 | | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

+ = plant infected. – = plant healthy. D = insect died. L = insect lost.
Superscript c = cast skin found, insect still a nymph.
Superscript a = cast skin found, insect now an adult.

the progeny of viruliferous parents before the progeny could acquire virus from infected plants. Three matings were made between known viruliferous individuals of the 6th generation of the active race (3). The number of plants infected by each parent varied from 1 to 4. In all, the 6 parents had infected 13 plants. Each female, after being mated, was transferred to an individual red clover plant (*Trifolium pratense* L.) suitable for the deposition of its eggs. It was transferred to a fresh plant about once a week. These plants were subsequently examined daily for nymphs, which were removed and placed individually on single crimson clover seedlings. In this way the nymphs were removed from the plant on which they were hatched usually before they were 24 hours old, occasionally after this age but before they were 48 hours old. Each of 121 surviving progeny was tested for 1 week on each of 7 successive crimson clover seedlings. Each test plant was observed in the greenhouse for 7 weeks after the insect was removed. None of the 847 test plants developed yellow dwarf. A few additional insects died before completing the test period of 7 weeks, but none of these infected any of the plants on which they fed. Transmission from parent to offspring did not occur.

Incidentally, in this experiment a record of all cast skins was kept. According to these records, 2 insects molted 6 times, 67 insects 5 times, 40 insects 4 times, 9 insects 3 times, and 1 insect twice in order to reach the adult stage. As some skins may not have been found, it is felt that the results on the lower number of ecdyses may not be reliable. However, it seems safe to conclude that the normal nymph has 5 instars, the same number reported earlier by Watkins (6).

The writer has thus far always obtained nonviruliferous nymphs when viruliferous adults were caged on alfalfa (*Medicago sativa* L. var. Grimm). This procedure has been used repeatedly with success when nonviruliferous progeny was desired. The results confirm the failure of the virus to pass from parent to young through the egg and indicate that alfalfa is either immune from the virus or very resistant to infection.

## SUMMARY

Clover leaf hopper nymphs in the 3rd, 4th, and 5th instars transmitted the potato yellow-dwarf virus in many instances. One record of transmission was obtained by a nymph in the 2nd instar.

No significant difference in mortality between infective and noninfective insects was observed.

The incubation period of the virus in crimson clover plants was variable. The majority developed symptoms in the 2nd and 3rd weeks after the feeding or inoculation period of one week. Some plants developed symptoms during the 1st week after the feeding period; others did not develop symptoms until the 5th week.

The incubation period of the virus in the insect was demonstrated to vary from 6 to 10 days. There is evidence that in individual insects the incubation period may be much longer.

Individual insects may infect crimson clover seedlings daily for at least 10 days. On the other hand, a lapse of many days between transmissions is common. In one case there was a 25-day interval between two transmissions by the same insect.

Insects transferred daily to fresh healthy clover plants remained infective for as long as 44 days. Insects fed on rye were still infective after 52 days.

Virus did not pass from parents to progeny through the eggs or sperms of the insect vector.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY,
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

## LITERATURE CITED

1. BLACK, L. M. Some insect and host relationships of the potato yellow-dwarf virus. (Abstract) Phytopath. 26: 87. 1936.
2. ———. A study of potato yellow dwarf in New York. Cornell Univ. Agr. Exp. Sta. Mem. 209: 1–23. 1937.
3. ———. Genetic variation in the clover leafhopper's ability to transmit potato yellow-dwarf virus. Genetics 28: in press. 1943.
4. FISHER, R. A. Statistical methods for research workers. 6th ed. Oliver and Boyd (London). 1936.
5. FUKUSHI, T. Transmission of the virus through the eggs of an insect vector. Proc. Imp. Acad. (Japan) 9: 457–460. 1933.
6. WATKINS, T. C. Clover leafhopper (*Aceratagallia sanguinolenta* Prov.). Cornell Univ. Agr. Exp. Stat. Bull. 758: 1–24. 1941.
7. YULE, G. U. An introduction to the theory of statistics. 6th ed. Charles Griffin and Co., Ltd. (London). 1922.

# AN ALTERNARIA DISEASE OF ZINNIA

A. W. Dimock and John H. Osborn

(Accepted for publication September 14, 1942)

In the summer of 1934 specimens of an undescribed leaf spot of zinnias (*Zinnia elegans*) were deposited in the herbarium of the Cornell department of plant pathology, Cornell University, by John Dearness, who had collected the specimens a few days earlier in a garden in Ontario, Canada. The organism associated with the spots was tentatively referred to *Alternaria solani,* though no detailed study of the disease or the organism was made at that time. During the same summer, what appeared to be the same disease was noted in the floriculture gardens at Cornell University by C. E. F. Guterman, who established by controlled experiment the pathogenicity of the Alternaria strain involved. Unfortunately, no account of this work was ever published. The disease has recurred annually in the Cornell gardens and has increased so greatly in severity that it is now planned to abandon further culture of zinnias in these gardens until adequate, practical control measures can be devised. During the past 3 years the writers have received specimens of zinnias affected by the disease from gardens in many sections of New York State and have seen the disease in severe form in a commercial planting near New York City.

Recently (1936–1940), a zinnia disease, apparently identical with that herein described, has been reported by Weber (10) and Neergaard (7) in Denmark, and by Beaumont and Staniland (5) in England. Gram and Rostrop (6), earlier (1923), reported a seedling blight of zinnia in Denmark caused by *Macrosporium caudatum.* It is possible that the same disease was here involved and that the pathogen was incorrectly identified. Alternaria on zinnia in the United States has been reported from Florida (1), Connecticut (2, 3), and New York (4), though it is not known whether the diseases reported from Florida and Connecticut are the same as the one in New York. In the autumn of 1941, two independent reports of limited development of the alternaria disease in zinnia plantings in California were received.[1] Shortly after this manuscript was originally submitted for publication, the senior writer was advised by S. P. Wiltshire of the Imperial Mycological Institute of a paper by Pape (9) in which the alternaria disease of zinnia and its pathogen are described. Inasmuch as Pape's paper may not be readily available to American plant pathologists for some time to come, and because the disease appears to be a potentially serious threat to zinnia culture, the following account is presented.

[1] Letter dated September 27, 1941, from Dr. Kenneth Baker, University of California at Los Angeles; verbal communication from Dr. R. C. Allen, Cornell University, following visit to the Pacific Coast in summer of 1941.

## SYMPTOMS OF THE ALTERNARIA DISEASE

### On Foliage

Perhaps the most common and conspicuous symptom of the alternaria disease is the spotting of the foliage. Individual spots are at first circular

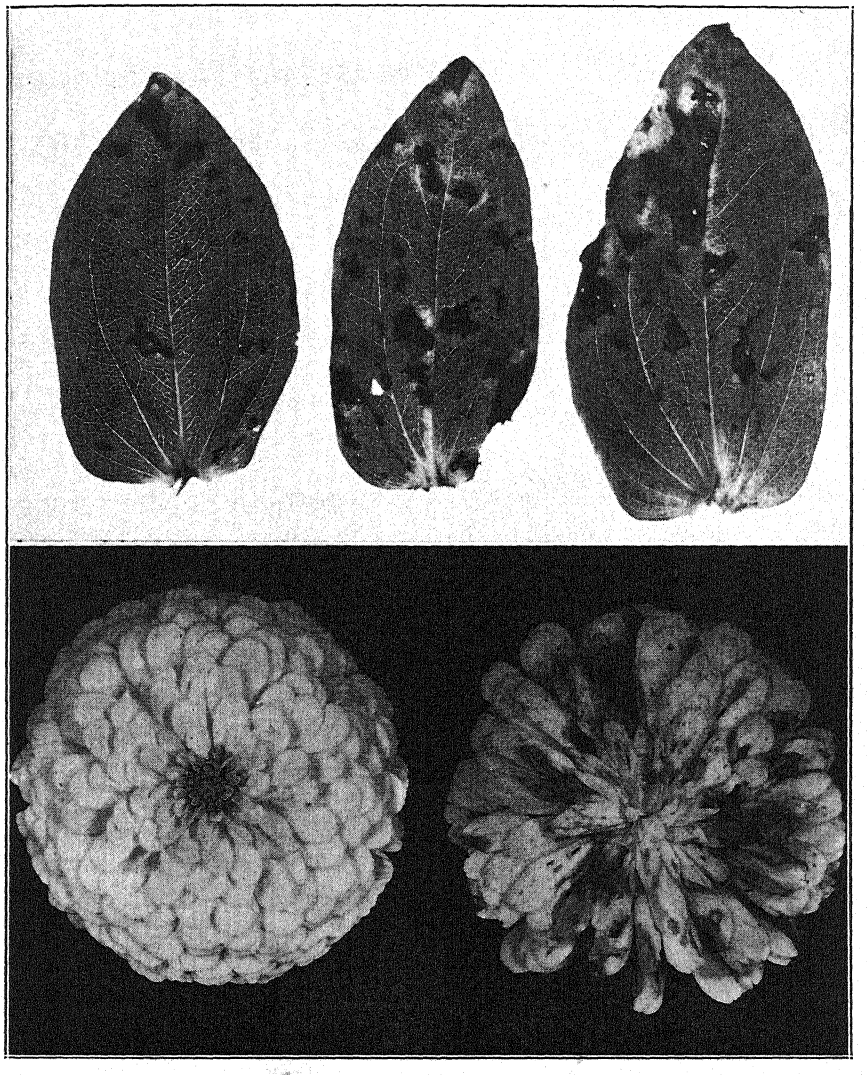


FIG. 1. Spotting of leaves and flower of white, dahlia-flowered variety of *Zinnia elegans* following natural infection with *Alternaria zinniae*.

in outline, but rapidly become irregular. The spots vary in size from 2 to 10 mm. in diameter (averaging about 5 mm.), are reddish-brown and may have greyish-white centers on the upper leaf surface (Fig. 1). Differentiation of margin and center is lacking on the lower surface. As the spots

increase in size and number, the affected leaves become brown and dry. Sporulation is often rather poor on leaf spots, but, under proper conditions, spores may be produced in abundance.

### On Blossoms

Blossoms may be very severely affected by the alternaria disease. Brown spots 1 or 2 mm. in diameter and sometimes having greyish-white centers appear on the petal tissues of the conspicuous ray flowers. Sporulation is often profuse on these spots and secondary infections may be abundant. Affected petals soon darken and wither, and the blossoms are rendered worthless as cut flowers or garden specimens (Fig. 1).

### On Stems

Numerous small reddish spots, sometimes with greyish white centers, usually are visible along the internodal areas of the stems of affected plants. These are rarely more than 1 mm. across, though they frequently show longitudinal elongation. Such spots are ordinarily superficial. Occasionally, through coalescence, rather large areas may be involved. Large lesions, frequently girdling the stems, often form at the nodes, due either to growth of the fungus inward from affected leaves or to infection occurring directly in the leaf axils. Unlike the internodal spots, the nodal lesions ordinarily do not remain superficial, and the distal portions of the affected stems may be killed by complete girdling at the nodes. Stem tissues often are invaded by growth of the fungus downward from badly affected blossoms. Basal cankers are common on the stems of plants in infected beds. These are dark brown to black and become sunken in the central portion. Fungous growth seems largely limited to the succulent cortical tissues, but complete girdling may occur. Affected plants often wilt completely, even when the basal cankers do not entirely girdle the stems. The root systems of such plants may remain healthy.

### On Roots

Root infection, while the least conspicuous, is perhaps the most serious phase of the disease since it is not amenable to control by the relatively simple measures that might be employed to control infection on stems, foliage and blossoms. The cortical tissues of affected roots become dark grey, rot completely, and slough off, resulting in wilting and death of the plants. Isolation of the pathogen from infected roots is rather difficult, since secondary organisms follow in rapidly.

## ETIOLOGY

Isolations made by various workers at Cornell during the past 7 years from typical lesions on zinnia leaves and stems have consistently yielded a rather uniform strain of a fungus of the genus *Alternaria*. The pathogenicity of this organism has been repeatedly proved by inoculation of healthy zinnias with mycelium and spores from pure cultures.

## Development in Leaf Spots

Microscopic examination of cleared infected leaf tissue reveals the presence of light-colored intra- and intercellular hyphae within the lamina, and brown-walled conidiophores pushing through the stomata (Fig. 2). Spores of the fungus usually are visible on fresh specimens when atmospheric conditions favor sporulation. Such spores always appear to be borne singly on the conidiophores, but occasionally, on material which has been kept for a few days in a dry chamber, catenulation of spores is evident. Chains of as many as 8 spores have been seen. The spores are large, averaging 170–210 by 20–24 microns, with a beak usually more than twice as long as the

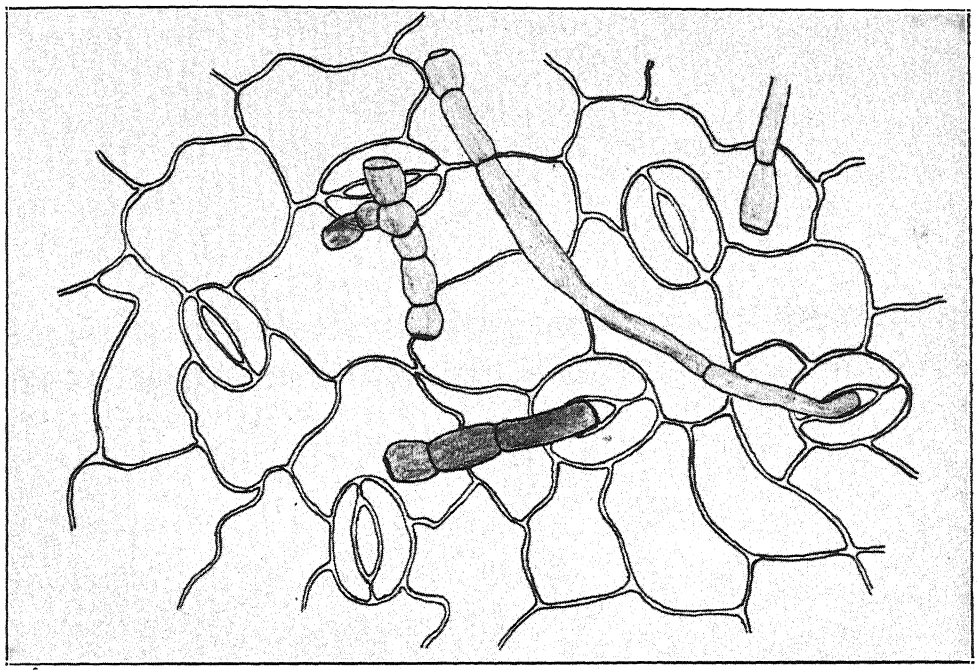


FIG. 2. Camera-lucida drawing of cleared portion of infected zinnia leaf showing conidiophores of *Alternaria zinniae* emerging through stomata.

body of the spore (Fig. 3, G). The body of the spore has numerous longitudinal and transverse septa. Stages in development of the spores are illustrated in figure 3.

## Growth in Culture

The fungus grows rather rapidly in culture and may cover a 9 cm. Petri dish of potato-dextrose-agar in 7 or 8 days at optimum temperature. The rough mat of mycelium, about 2 mm. high, may have a narrow whitish margin, but the central growth rapidly assumes a dark grey color. A reddish pigment diffuses into the medium as the culture ages. The formation of this reddish pigment has been demonstrated by the junior writer to be

dependent upon the presence of sugars in the culture medium. Sporulation has been very poor on all culture media tested, including potato-extract agar, potato-dextrose agar, bean agar, oatmeal agar, pea agar, cornmeal agar and water agar. The optimum temperature for growth of the organism was found to be about 27° C. (81° F.).

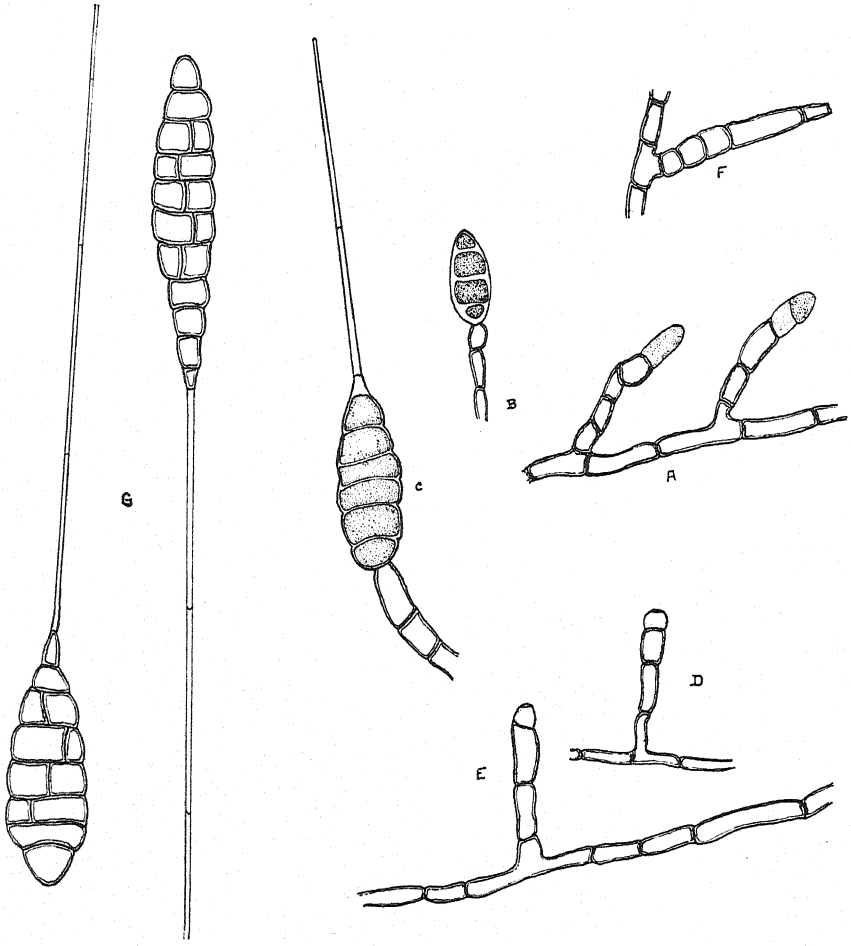



FIG. 3. Camera-lucida drawing showing development of conidia. A–C. Formation of conidia from terminal cell of conidiophores; material from potato-dextrose-agar culture. D–F. Conidiophores after abscission of conidia. G. Mature spores from naturally infected leaf.

## The Pathogen

The zinnia pathogens described briefly by Weber (10), Neergaard (7) and Beaumont and Staniland (5) appear to be the same as that herein described. Furthermore, S. P. Wiltshire, in correspondence with the senior writer, has expressed the opinion that the fungus described by Pape (9) as *Alternaria zinniae* n. sp., is identical with ours. We may therefore conclude that the alternaria disease of zinnias, in both Europe and North America, is caused by *Alternaria zinniae* Pape.

### PATHOGENICITY STUDIES

Although the pathogenicity of the Alternaria strain had been repeatedly proved by Cornell workers since 1934, a more detailed study of inoculation and infection was undertaken by the junior writer during the 1940–41 season. In order to avoid the variation which might result from the use of potentially variable spore inoculum taken from naturally infected field material, it was decided to use spore inoculum developed in culture from a single-spore isolate of the pathogen. Inasmuch as sporulation was very poor on all culture media tested and could not be induced by any of the methods commonly used for that purpose, a more effective method was sought. The following method proved satisfactory, although consistent results were not always obtained.

A dilute suspension of spores and mycelium in cooled, sterile 1 per cent water agar was poured in a thin layer in sterile Petri plates. Cheesecloth discs, previously cut to fit the plates and sterilized with hot air, were then placed three-deep on the surface of the agar in each plate, this operation being performed under a transfer hood. Moisture given off from the agar was absorbed by the lower discs of cheesecloth, leaving the upper disc comparatively dry. Spores were produced in abundance on the upper discs in 11 or 12 days and were readily removed by scraping in sterile water. The spores settled rapidly and the excess water was decanted without centrifuging. Spore inoculum thus obtained was applied in the leaf-infection study described below.

#### Relation of Time, Temperature, and Surface of Leaf to Incubation and Infection

The following experiment was designed primarily to determine the effect of temperature on establishment of infection and duration of incubation period, and, incidentally, to determine whether invasion occurs more readily through the upper or the lower leaf surface. Young zinnia plants, about 5 weeks old and approximately 4 inches tall, each bearing 2 pairs of fully expanded leaves, were used. Since trials at different temperatures had to be performed consecutively rather than concurrently, the actual age of plants used varied slightly.

The plants were marked for inoculation by imprinting 6 circles of india ink, about 5 mm. in diameter, on the upper surface of one leaf of each pair, and similarly imprinting 6 circles on the lower surface of the other leaf of each pair. There was thus a total of 12 upper-surface areas and 12 lower-surface areas marked on each plant. One drop of spore suspension was placed, by means of a finely drawn out pipette, on each marked area. The inoculum used for each temperature series was adjusted so that from 9 to 12 spores were present in each drop.

Six plants were used in each temperature series, the plants being placed in the constant-temperature infection chamber immediately following inoculation. One plant was then removed from the chamber every 6 hours

and placed on the greenhouse bench. Artificial means were not employed to hasten drying of the surface moisture, since observation indicated that natural drying was extremely rapid. Examinations were made periodically for 3 weeks, but after the second week no new infections appeared. The data are summarized in table 1.

These data indicate that the optimum temperature for infection lies above 65° F. and below 75° F., probably about 70° F. It is interesting to note that this is considerably below 81° F., the optimum for growth of the fungus on potato-dextrose agar. The data also indicate that the incubation period may be as short as 12 hours at 70–75° F., though consideration of the data for all temperature tests suggests that, at the temperatures studied, maximum infection is not reached with an incubation period of less than 24 hours. It has been the senior writer's experience that, unless artificially

TABLE 1.—*Effect of temperature, duration of exposure and leaf surface inoculated upon infection of leaves of Zinnia elegans by Alternaria zinniae*

| Temp. | Surface | Duration of exposure in hours | | | | | | Total No. lesions |
|---|---|---|---|---|---|---|---|---|
| | | 6 | 12 | 18 | 24 | 30 | 36 | |
| 65° F. | Upper | ..... | ..... | ..... | ..... | 6 | ..... | 14 |
| | Lower | ..... | 1 | ..... | 5 | 2 | ..... | |
| 70° F. | Upper | ..... | ..... | 1 | 7 | 8 | 2 | 56 |
| | Lower | ..... | 4 | 1 | 12[a] | 10 | 11 | |
| 75° F. | Upper | 1 | 3 | 3 | 2 | ..... | 3 | 38 |
| | Lower | ..... | 4 | 3 | 6 | 6 | 7 | |
| 80° F. | Upper | ..... | ..... | 5 | 3 | 2 | 1 | 22 |
| | Lower | ..... | ..... | 3 | 3 | ..... | 5 | |
| Total No. lesions ..... | | 1 | 12 | 16 | 38 | 34 | 29 | 130 |

Total No. lesions, upper surface—47; lower surface—83.
[a] 12 lesions represent 100 per cent infection.

dried, moisture sufficient to permit germination and infection may remain on the leaf surface for a number of hours after it appears to the eye to be quite dry. Conclusions concerning the precise duration of the incubation period should not be drawn without further study. It is evident that invasion occurred more readily through the lower surface of the leaf than through the upper surface. No attempt was made to determine whether this was correlated with number of stomata, thickness of cuticle, microclimate, or other factors.

## Stem and Root Inoculations

The following experiment was undertaken to test the pathogenicity of *Alternaria zinniae* to roots of zinnia plants. Fifteen 1-month-old zinnia seedlings were transplanted from seed flats to individual 3-inch pots of sterilized soil that had been infested by mixing in about a tablespoonful of alternaria inoculum. The inoculum had been prepared by growing the fungus in flasks of unmodified, sterilized moist greenhouse soil, then thoroughly mixing the soil-mycelium mass before using. Fifteen seedlings were

also transplanted to pots of sterilized soil which had been infested with inoculum of a Phytophthora isolate frequently obtained from decayed zinnia roots. In addition, 15 seedlings were inoculated with an isolate of Fusarium commonly obtained from decayed zinnia roots. Inoculation in this case was made by dipping the roots of the seedlings in spore suspension just prior to planting in the pots of sterilized soil. As controls, 15 uninoculated seedlings were planted in pots of noninfested sterilized soil. The roots of these seedlings had first been dipped in sterile water.

Within 12 days after inoculation, 7 of the plants of the alternaria series had died, showing typical root rot and stem decay. Only 4 of the plants of this series were alive after 6 weeks, and only one of these was in vigorous condition. None of the plants in the Phytophthora, Fusarium or control series became infected. Many of the plants of the Phytophthora series had remained somewhat stunted for 2 or 3 weeks, and had exhibited marginal scorching of the lower leaves, but all were definitely recovering after 6 weeks. *Alternaria zinniae* was successfully reisolated from roots of several of the dead plants in the alternaria series.

As a further check on the susceptibility of stem tissues, seven of the healthy survivors from the above experiment were inoculated by placing agar-mycelium inoculum of *Alternaria zinniae* in slit wounds made in the stems. Uninoculated slit wounds were similarly made in a like number of the healthy plants to serve as controls. Typical Alternaria lesions had developed on all 7 inoculated plants within 2 weeks and reisolation attempts were successful. All of the controls remained healthy. These tests conclusively demonstrated the ability of *A. zinniae* to infect the roots and stems of zinnia.

### PERPETUATION AND DISSEMINATION OF THE PATHOGEN

Intensive studies of the perpetuation and dissemination of the pathogen have not been undertaken. Limited studies and circumstantial evidence, however, have demonstrated rather well that the pathogen may live over at least one winter on infested plant débris (or perhaps independently) in the soil or on its surface. The annual recurrence of the disease in Cornell plantings strongly suggested carry-over in the soil; but more conclusive evidence was obtained during the past season when half of a lot of seedlings was shipped to another location for planting in a private garden and the remainder kept for planting in the Cornell gardens. No evidence of the disease appeared in the new locality, whereas the entire planting in the Cornell gardens was destroyed by the disease before the first of July. It was further indicated in the above test that dissemination of inoculum is readily effected by means of cultivating tools, or the washing of soil, or both. The beds in which the Cornell planting was made had not been occupied by zinnias for over 4 years and were about 100 feet distant from the beds in which zinnias had been planted for the past 4 or 5 years. They were, however, prepared and cultivated with the same tools as the old infested beds and were several feet lower in elevation, so that some soil washing may well

have occurred. These modes of dissemination, while important in localized intensification of the disease, are probably of no consequence in its long-distance spread.

Available evidence strongly indicates that the pathogen is seed-borne. As noted above, blossom infection is usually severe whenever the foliage is infected. Sporulation on infected petal tissue is very abundant and surface contamination of seed harvested from infected blossoms would be inescapable. The following test, conducted by the junior writer, is suggestive that actual infection of the seedcoat may occur and result in dissemination of the pathogen as internal mycelium. Thirty-five zinnia seeds (var. Giant Mammoth Lavender) were taken at random from a seed packet obtained from a popular commercial seedhouse. These were surface-sterilized for a short period in 1–1000 mercuric chloride solution, washed in sterile water, and plated out on potato-dextrose agar. An Alternaria, indistinguishable culturally from typical leaf spot isolates, was obtained from 21 of these seeds. Inoculation tests proved this Alternaria isolate to be pathogenic to zinnia foliage, producing typical Alternaria lesions. A second test, with seeds of the same source and variety but from a different packet, failed to reveal the presence of Alternaria. Additional evidence that the fungus is seed-borne is offered by Neergaard (8), who reported good control of what is presumed to be the same disease by seed treatment with one of the organic mercury compounds (0.25 per cent Germisan).

## CONTROL

Inasmuch as it seems evident that the pathogen may readily be borne in or on the seed, it is of first importance that flower-seed producers become acquainted with the disease, recognize its potential importance, and attempt to prevent its development in their plantings. Thus far, the disease appears to be of limited occurrence both in the seed-producing areas and in private plantings. It is obvious, however, that if the disease ever were to develop seriously in the seed fields, it would soon become widely distributed. It is possible that the comparatively rainless summers prevalent in the seed-producing areas on the Pacific Coast have provided and will continue to provide a natural check on the disease. The matter would bear investigation.

No study of special control methods for this disease has yet been undertaken, but the following logical suggestions are offered:

1. Seed should be treated with a fungicide (mercuric chloride dip, Semesan, cuprous oxide, or other) to reduce the hazard from inoculum borne on the seed surface.
2. Thorough field and garden sanitation should be practiced.
3. The longest possible rotation of field planting site should be employed.
4. Cultivating tools should be cleaned, and preferably sterilized, before using in new zinnia planting areas.
5. Seedlings and young plants should be treated frequently with a good protective fungicide, making an effort to obtain coverage of the lower surface of the leaves.

SUMMARY

A disease of garden zinnia, *Zinnia elegans* Jacq., causing spotting of the petals, foliage and stems, and rotting of the roots, has been under observation at Cornell University since 1934.

The disease is caused by *Alternaria zinniae* Pape.

Conidia of *A. zinniae* averaged 20–24 by 170–210 μ including beak, the beak commonly being twice as long as the body of the spore. Catenulation has not been observed under natural conditions.

The optimum temperature for growth of the fungus in culture was found to be about 27° C. (81° F.), whereas the optimum temperature for leaf infection was found to be about 70° F.

An incubation period of approximately 24 hours was required for the maximum development of leaf infection.

Invasion occurs more readily through the lower surface of the leaf.

Evidence indicates that the pathogen may be seed-borne and may survive at least one winter in or on the soil.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY,
ITHACA, NEW YORK.

LITERATURE CITED

1. ANON. U.S.D.A. Pl. Dis. Rptr. Suppl. 42: 367. 1925.
2. ————. Ibid. Suppl. 96: 289. 1936.
3. ————. Ibid. Suppl. 103: 244. 1937.
4. ————. Ibid. Suppl. 119: 279. 1939.
5. BEAUMONT, A., and L. N. STANILAND. Fifteenth Ann. Rept. Dept. Plant Path., Seale-Hayne Agric. Coll., Newton Abbot, Devon, for year ending Sept. 30, 1938. p. 35. 1938.
6. GRAM, E., and SOFIE ROSTRUP. [Survey of the diseases of cultivated agricultural and horticultural plants in 1922.] Tidsskr. Planteavl. 24 (2): 236–307. 1923.
7. NEERGAARD, P. [Annual report of the phytopathological laboratory of J. E. Olsen's widow from April 1, 1936, to March 31, 1937.] 11 pp. 1937.
8. ————. [Annual report of the phytopathological laboratory of J. E. Olsen's widow, Seed Growers, Copenhagen, from April 1, 1939, to March 31, 1940.] 13 pp. 1940.
9. PAPE, H. Die Alternaria-Krankheit der Zinnie und ihre Bekämpfung (*Alternaria zinniae* n. sp.). Angewandte Botanik 24 (2): 62–79. 1942.
10. WEBER, ANNA. [Plant diseases in Denmark in 1936. Survey of data collected by State Phytopathological Exp. Stat.] Tidsskr. Planteavl. 42 (2): 189–249. 1937.

# A DESCRIPTION OF THE FUNGUS CAUSING COTTON RUST, AND A PRELIMINARY SURVEY OF ITS HOSTS

JOHN T. PRESLEY AND C. J. KING

(Accepted for publication August 12, 1942)

## INTRODUCTION

The fungus *Aecidium gossypii* E. and E., causing rust of cotton in the Southwestern States, has been designated by Arthur and others as synonymous with *Puccinia schedonnardi* Kell. and Swing.[1] In 1941 the senior writer reported the establishment of a connection between *Aecidium gossypii* and a Bouteloua rust.[2] The rust was tentatively determined as *Puccinia boutelouae* (Jennings) Holw., and specimens were sent to Purdue University for confirmation. The material was examined by G. B. Cummins whose findings indicate that the rust is not *P. boutelouae,* but probably an undescribed species with at least one character distinct from all other rusts found on *Bouteloua* spp. The outstanding difference is the occurrence of 3 equatorial pores in the urediospores of the new species rather than several scattered pores, as listed for the other Bouteloua rusts. Recently, photomicrographs have been made to show the presence and arrangement of pores of the urediospores, and these are in accord with the findings of Cummins. (See Fig. 2, B.)

Under greenhouse conditions 38 American commercial varieties of cotton proved susceptible to the rust in all stages of growth when inoculated with germinating telia. Very young cotton seedlings sometimes were killed when the sporidial shower was especially heavy. The hypocotyl, coytledons, and true leaves became completely covered with pycnial clusters, and the plants soon died. However, seedlings survived a moderate sporidial shower and produced aecial clusters on all exposed parts of the plant. Of the 3 Asiatic varieties of *Gossypium arboreum* L. tested, the var. *assamica* Watt and var. *sanguineum* (Hassk.) Watt were resistant; while the red-flower var. *nanking* (Meyen) Harland was mildly susceptible, and produced abundant anthocyanin around the pycnial clusters.

## FIELD INFECTION

Under field conditions in Arizona the rust attacks the cotton crop immediately following the first summer rains. The time of attack varies according to rainfall and humidity but usually occurs in July. The severity of attack depends upon weather conditions and the amount of inoculum present. Ordinarily, the first attack is relatively light and may pass unnoticed, but with each succeeding rain additional infection occurs. Greenhouse experiments have shown that telial material will produce infection continu-

[1] Arthur, Joseph Charles. Manual of the Rusts in United States and Canada. pp. 143–144. 1934.

[2] Presley, John T. *Aecidium gossypii.* The aecial stage of *Puccinia boutelouae.* Phytopath. 32: 97. 1941.

ously for at least 12 days. Several series of cotton seedlings were passed through an incubator, without changing the inoculum, on grass suspended above the seedlings. The last series, though not so heavily infected as some of the first series, showed typical pycnial clusters, and aecia developed from them.

The first symptoms of rust on the cotton plants are small orange-color spots on the upper surface of the leaves (Fig. 2, C). These spots contain the pycnia (Fig. 2, A) and soon become discolored. The pycnial ooze becomes brownish with age and anthocyanin is produced by the leaf around

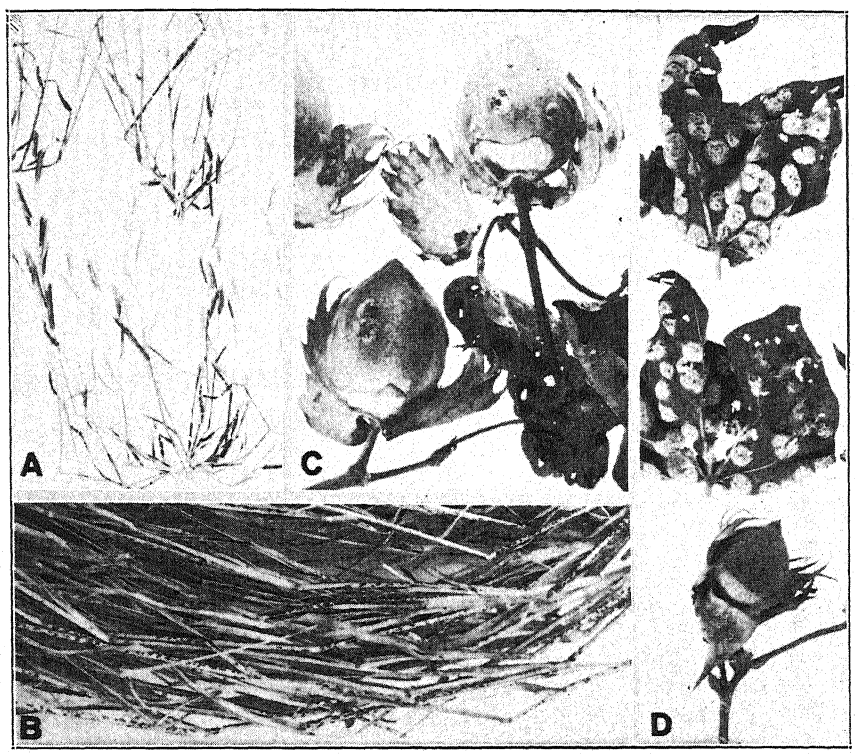



FIG. 1. A. Rust-infected grass (*Bouteloua rothrockii*) collected from uncultivated area; (B) from an irrigated cotton field. Somewhat reduced. C and D. Rust spots on cotton leaves, bracts and bolls. Note pycnia on upper surface of leaves and on bolls in C and aecial spots on under surface of leaves and bracts in D. Less than ½ nat. size.

the pycnial cluster (Fig. 1, C). Within a week aecia begin to form on the under surface of the leaf. The aecia are formed in circular spots surrounding the pycnia, and vary in size from 0.2–0.3 mm. in diameter (Fig. 2, F). At first the aecial clusters are deep yellow to orange, but, on exposure to the sun, soon fade and appear light-yellow to colorless (Fig. 1, D).

The shady, moist conditions in a cotton field favor the development of the rust on grass. The telia form soon after the uredia and mature early. Specimens on grass collected in late July proved viable and ready to reinfect cotton with the first favorable weather conditions.

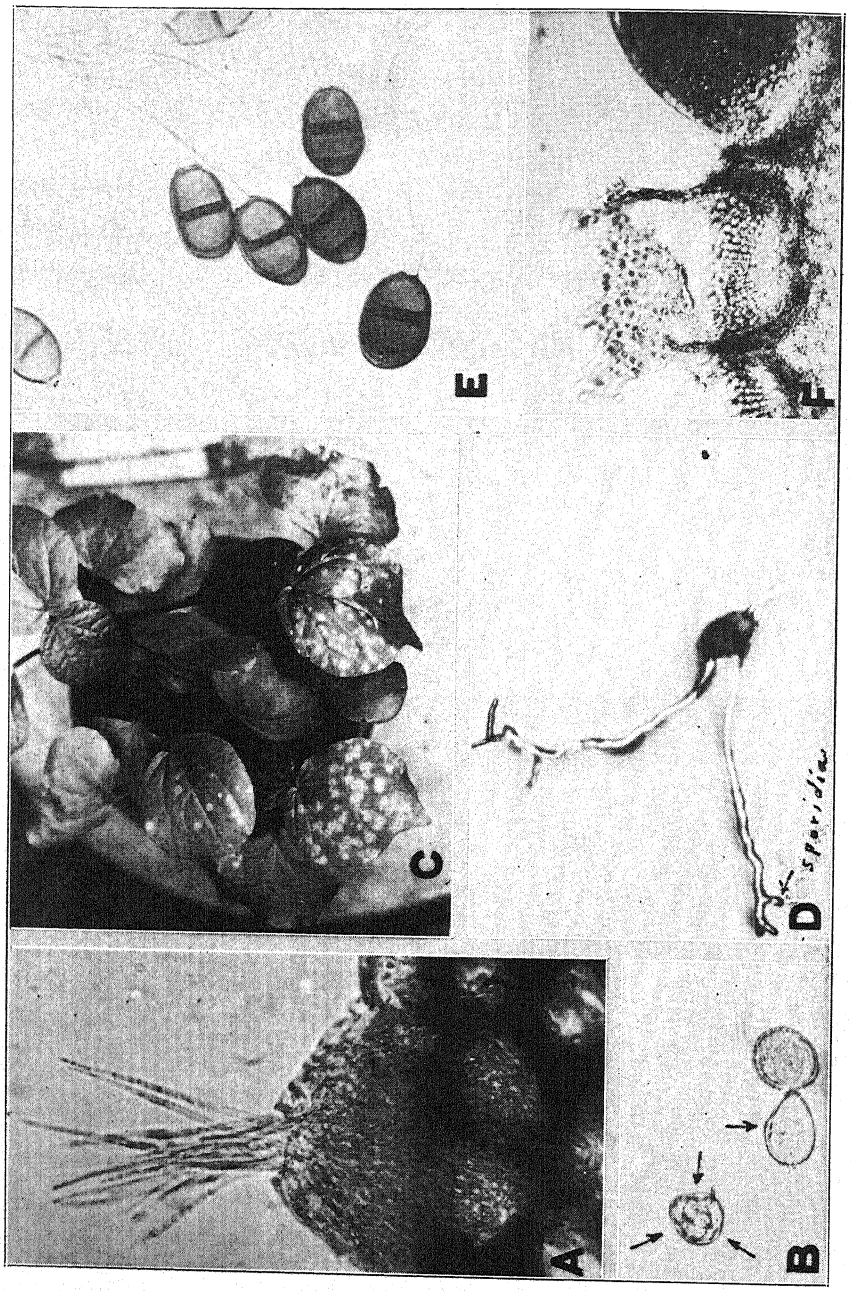



FIG. 2. A. Pycnium of the cotton rust fungus. ×400. B. Urediospores. Upper spore (vertical view). Note 3 pores shown in upper spore. Middle spore (horizontal view), note equatorial arrangement of pores. ×400. C. Pycnial spots on leaves of cotton seedlings after exposure to shower of teliospore from infected grass suspended above. ×about ½. D. Germinating teliospore showing promycelium emerging from both cells and developing sporidia on appendages near the ends. ×300. E. Teliospores. ×400. F. Cross section of aecia showing peridium and aeciospores. ×200.

The uredial stage of the fungus is rather inconspicuous, since it is formed largely on the leaves of the grass. The telia on the other hand are very conspicuous, sometimes covering the grass stems for several inches with prominent, dark pustules, which also appear among the uredia on the leaves. The teliospores are of the typical Puccinia type with two cells (Fig. 2, E). Both cells may germinate to form promycelia from which arise the sporidia (Fig. 2, D).

DESCRIPTION

The following description of the rust organism is based on pycnial and aecial material collected on cotton (*Gossypium hirsutum*) in a cultivated field at Hidden Valley, Arizona, in October, 1941. The telial and uredial materials were collected at the same time from *Bouteloua rothrockii* Vasey, growing adjacent to the cotton. A portion of the material from the same collection was submitted to Cummins and other portions were used to obtain infection on cotton seedlings in greenhouse tests.

### Puccinia stakmanii n. sp. Presley

O. Pycnia amphigenous, also caulicolous, numerous, in small, sometimes discolored, slightly raised circular groups, crowded, punctiform, honey-yellow to cadmium-orange, becoming brownish, depressed, globose, 90–120 μ in diameter; ostiolar filaments 60 to 100 μ long.

I. Aecia chiefly hypophyllous, also caulicolous, in irregular groups surrounding the pycnia, 2–5 mm. in diam., somewhat crowded, cylindric, 0.4–1.0 mm. high, 0.2–0.3 mm. wide; peridium at first orange becoming yellowish or colorless, the margin lacerate and recurved; peridial cells quadratic or oblong, 17–30 × 11–20 μ, overlapping, the outer wall 6–9 μ thick, striate, the inner wall 2.5–4.0 μ thick, strongly echinulate; aeciospores globoid or broadly oblong, 13–19 × 18–25 μ; wall very pale yellow to colorless, 2–5 μ thick, finely verrucose, often appearing smooth.

II. Uredia mostly epiphyllous, internerval, oblong or linear, becoming confluent, 0.3 to several mm. long, early naked, pale cinnamon-brown, pulverulent, the ruptured epidermis apparent; urediospores globoid to broadly ellipsoid, 18–22 × 25–27 μ, the wall pale cinnamon-brown, 1.5–2.5 μ thick, moderately echinulate, the pores equatorial, usually 3.

III. Telia amphigenous and caulicolous, numerous, often crowded, rounded, elliptic or linear, becoming confluent, 0.3 to several mm. long, early naked, the ruptured epidermis at first conspicuous; teliospores oblong or broadly ellipsoidal, 19–25 × 26–38 μ, rounded at both ends or sometimes obtuse above, only slightly if at all constricted at the septum, this sometimes oblique or vertical; wall dark chestnut-brown, 1.5–2.5 μ at the apex 4–9 μ thick; pericel colorless, twice or thrice the length of the spore, sometimes attached at an angle.

Habitat: Pycnia and aecia on living leaves and stems of *Gossypium barbadense, G. hirsutum*, and many other species of *Gossypium* by inoculation; uredia and telia on living leaves and culms of *Bouteloua rothrockii* and other species of *Bouteloua* by inoculation; Arizona and Texas.

### Puccinia stakmanii sp. nov. Presley

O. Pycnia amphigena etiam caulicola numerosa depressa gilva vel aurantiaca globosa diametro 90–120 μ in maculis parvis circularibus leviter elevatis.

I. Aecia praecipue hypophylla cylindrata alta 0.4–1.0 mm. lata 0.2–0.3 mm. maculis disparibus diametro 2 usque ad 5 mm; peridium aurantiacum deinde lutescens margine lacerato recurvato; sporae sphaeroidae vel late oblongae 13–19 × 18–25 μ.

II. Uredia plerumque epiphylla internervalia oblonga vel linearia longa 0.3 usque ad plura mm mature denudata; sporae sphaeroideae vel late ellipsoideae 12–22 × 25–27 μ pariete subfusco crassitudine 1.5–2.5 μ foraminibus equatorialibus plerumque 3.

III. Telia amphigena et caulicola numerosa saepe aggregata longa 0.3 usque ad plura mm mature denudata; sporae oblongae vel late ellipsoideae 19–25 × 26–38 μ septo aliququando oblique vel perpendiculare; pediculus non coloratus longitudine sporam duplo usque ad triplo excedens.

Type specimens are deposited in the Mycological collections of the Bureau of Plant Industry.

## SURVEY OF THE HOST RANGE

The rust whose aecial stage occurs on all the principal varieties of both the upland and American-Egyptian cotton varieties grown commercially in the irrigated Southwest causes considerable damage in some seasons in limited areas. The telial stage of the rust occurs on species of *Bouteloua*. These grasses are commonly found in Texas, New Mexico, Arizona, and California. The continued expansion of the cotton acreage on virgin lands in the States where these grasses are native may bring further reports of damage by the disease.

Since cotton only recently was discovered to be the aecial host of the Bouteloua rust, it appeared desirable to make a preliminary survey of the plants susceptible to this stage of the fungus, as well as to explore the possibility of finding resistance in some cultivated variety of cotton. The survey was conducted in part at the U. S. Field Station, Sacaton, Arizona, and in part at the University of Minnesota in connection with the Division of Plant Pathology. Some interesting facts were disclosed by these studies, among them being the host range of the fungus. In the tests so far it has been possible to produce the aecial stage only on the genus *Gossypium*, and the uredial and telial stages only on the genus *Bouteloua*. It should be borne in mind, however, that the results here reported were obtained from tests on plants that were grown and inoculated under greenhouse conditions. Although differences were observed in susceptibility and resistance among the seedlings, the mature plant reaction under field conditions is known only for the 4 or 5 varieties of cotton grown commercially in the Southwest.

## METHOD OF INOCULATION AND RESULTS

In making the inoculations the usual procedure used in cereal-rust investigations was followed. The telial material on bunches of grass was presoaked and suspended above the cotton seedlings in an incubator for 3 or 4 days. The cotton plants were then transferred to the greenhouse where they were placed under conditions of maintained high relative humidity. The pycnia usually appeared on the leaves and stems in 4 to 6 days following removal from the incubator. There was a wide variation in the number of pycnial clusters formed on any one leaf, since it is necessary for the sporidia to fall and penetrate the leaf tissue in order to produce infection. Apparently, there is no way to control accurately the number of sporidia that will fall and germinate on a particular surface, hence the criterion of susceptibility applied in the classification is rather arbitrary. If both pycnia and aecia were abundant the plant was considered susceptible. If only pycnia were formed the plant was considered mildly susceptible, and if there were neither pycnia nor aecia the plant was considered resistant. The different series of cotton plants subjected to inoculation were run either in duplicate or triplicate. A list of the cottons and other malvaceous and graminaceous plants tested and their classification according to resistance or susceptibility to artificial infection are given below.

LIST OF MALVACEOUS AND GRAMINACEOUS PLANTS, CLASSIFIED ACCORDING TO THEIR RESISTANCE OR SUSCEPTIBILITY TO ARTIFICIAL INFECTION FROM THE COTTON RUST FUNGUS

## Plants Exposed to Germinating Telia

### I. Cultivated Cottons

*Gossypium hirsutum* L.

| | | |
|---|---|---|
| ***Acala (several strains) | ***Durango | ***Oklahoma Triumph |
| " Ballard | " Ewing's Long Staple | " Paris Big Boll |
| " Burnett | " Half and Half | " Petty's Toole |
| " Cleveland (several strains) | " Hartsville | " Rowden |
| " Coker's (several strains) | " Holden | " Sikes |
| " Columbia | " Kasch | " Spear's Upland |
| " College | " Kekchi | " Stoneville (several strains) |
| " Cooks | " Lone Star | " Texas Special |
| " D & P L (several strains) | " Meade | " Trice |
| " Davidson's Sunshine | " Mebaken | " Toole Perry |
| " Delfos (several strains) | " Mexican Big Boll | " Tuxtla |
| " Delta type Webber | " Miller | " Westex |
| " Dixie Triumph | " Missdel (several strains) | |

| | | |
|---|---|---|
| *G. barbadense* L. | | |
| ***Peruvian | ***Sakellaridis | ***Sea Island |
| " Pima | " S × P (Sakel × Pima) | *Zagora |
| *G. arboreum* L. | | |
| **Garo Hill | *Sanguineum | **Nanking |
| *G. hopi* Lewton | | |
| ***Hopi | | |

### II. Cotton, Wild Species

| | | |
|---|---|---|
| **G. *anomalum* Wawra et Peyr. | **G. *harknessii* Brandg. | **G. *sturtii* F. v. M. |
| **G. *aridum* (Rose and Standl.) Skovsted | **G. *klotzschianum* Anders. | ***G. *thurberi* Tod. |
| **G. *armourianum* Kearney | **G. *stocksii* M. Mast. | ***G. sp. (Florida wild) |
| **G. *davidsonii* Kell. | | |

### III. Other Malvaceae

| | | |
|---|---|---|
| *Althaea rosea* Cav. | *H. *militaris* Cav. | *Malva *parviflora* |
| *Hibiscus *esculentus* L. | *H. *rosa-sinensis* L. | |

## Grasses Inoculated with Aecia from Cotton

| | |
|---|---|
| ***Bouteloua *aristidoides* (H.B.K.) Griseb. | *M. *rigens* (Benth.) Hitchc. |
| ***B. *barbata* Lab. | *M. *wrightii* Vasey |
| **B. *curtipendula* (Michx.) Torr. | *Sporobolus *airoides* (Torr.) Torr. |
| ***B. *gracilis* (H.B.K.) Lag. | *S. *asper* (Michx.) Kunth |
| ***B. *rothrockii* Vasey | *S. *contractus* Hitchc. |
| *Muhlenbergia *arenacea* (Buckl.) Hitchc. | *S. *cryptandrus* (Torr.) A. Gray |
| *M. *monticola* Buckl. | *S. *flexuosus* (Thurb.) Rydb. |
| *M. *porteri* Scribn. | *S. *giganteus* Nash |
| *M. *pungens* Thurb. | *S. *heterolepis* A. Gray |
| *M. *racemosa* (Michx.) B. S. P. | *S. *texanus* Vasey |
| *M. *repens* (Presl) Hitchc. | *S. *wrightii* Munro |

*** = Susceptible
** = Mildly susceptible
* = Resistant

It is of interest to note that all of the Old World cottons included in the test were either resistant or mildly susceptible, whereas most of the New World cottons were readily attacked. The Old World cottons have 13 chromosomes, and the cultivated New World cottons have 26. The question now arises, is there a relation between genetic make-up of the plant and susceptibility to the rust fungus? If we examine the table of plants tested we shall see that several American wild cottons were included, some of them having 13 chromosomes, others 26. The 26-chromosome group of cottons, both wild and cultivated, was susceptible without exception, while the 13-chromosome group was only mildly susceptible, with the exception of *G. thurberi*. This species was one of the most susceptible of the plants tested, based upon the number of pycnial clusters formed. Leaf texture and size undoubtedly could play an important role in an experiment of this nature.

It seems reasonable to believe that some of the species considered here as mildly susceptible might, under more favorable conditions, appear quite susceptible, and, conversely, some species, apparently rather susceptible under the conditions of the experiment might seem resistant under different conditions. Considering the results obtained with cotton there would appear to be little if any correlation between plant type or growth habits and susceptibility. There seems, however, to be some connection between the genetic make-up of the plant and degree of susceptibility.

### INCIDENCE AND DAMAGE TO THE COTTON CROP

Ordinarily, the rust fungus attacks the leaves of cotton and may cause moderate shedding without seriously impairing the plants. If, however, there occurs a period of frequent rains accompanied by high relative humidity during late summer or early fall, the infection may become so severe that most of the leaves and many of the bolls are shed. An infection of such severity occurred in 1940 at Hidden Valley, Arizona.

The damage caused by a severe rust epidemic is difficult to estimate in terms of dollars or bales of cotton lost. It is apparent, however, that the cotton plants are weakened by the defoliation, and many bolls, not actually killed by the fungus, are left in condition to become easy prey to some of the boll-rotting organisms. Brown and Streets,[3] from observations made in 1930 in the Santa Cruz Valley, south of Tucson, Arizona, estimated loss of cotton yields as high as 75 per cent. The writers have observed fields in which the infection reached 100 per cent, and where more than half of the effective leaf surface of the plants was destroyed or impaired by the fungus. In such fields the loss in yield undoubtedly is heavy.

It is indicated from these studies that the telial stage of the rust occurs on certain species of *Bouteloua*, which, under ordinary desert conditions, are small and grow only during the rainy reason in summer or fall. In cotton fields where water is supplied by irrigation the same grasses will grow all summer and attain a size many times that of desert-grown specimens. A

[3] Brown, J. G., and R. B. Streets. Diseases of Field Crops in Arizona. University of Arizona Bull. 148: 85–228. 1934.

comparison of *Bouteloua* plants grown on desert land adjacent to cotton and in a cultivated field is shown in figure 1, A and B. Not only is there a great difference in size of plants in irrigated and nonirrigated areas, but, also, in the amount of inoculum produced.

Since all species of *Bouteloua* commonly present in the irrigated Southwest are susceptible to the rust, there is a good chance of an abundance of telial material being present to infect cotton planted in any newly cultivated area. A greater amount of inoculum, however, is produced in the cotton fields where the grass is permitted to grow all summer. This is particularly true in fields where the cotton has been rattooned or "stubbed" and where clean culture is difficult to maintain. Prevention and removal of the grasses by clean cultural practices within the fields and along the ditch banks and fences should be beneficial as a control measure.

## SUMMARY

The fungus causing the rust of cotton in the Southwestern States differs in at least one taxonomic character from the rust species heretofore described on *Bouteloua*. Within its urediospores 3 equatorial pores occur instead of several scattered ones, commonly present in other Bouteloua rusts. The name *Puccinia stakmanii* is proposed to designate this form of rust as a new species.

In a preliminary survey of the host range by greenhouse infection methods the following varieties and species were exposed to germinating telia: 48 cultivated cotton varieties representing 4 species, wild cottons representing 10 species, and other malvaceous plants representing 5 species. All cultivated cottons represented in *Gossypium hirsutum, G. barbadense,* and *G. hopi* were classed as susceptible; of the 3 varieties represented in *G. arboreum* 2 were resistant and 1 mildly susceptible; and of the 10 wild cotton species 8 were mildly susceptible and 2 susceptible; the other 5 malvaceous species were classed as resistant.

Twenty-two species of grasses representing 3 genera were inoculated with aecial material from infected cotton. From the results these were classed as follows: 4 of the 5 species of *Bouteloua* tested were susceptible and 1 mildly susceptible; the 8 species of *Muhlenbergia* and 9 species of *Sporobolus* inoculated were resistant.

The incidence and severity of rust infection on cultivated cotton are dependent on conditions of summer rainfall and humidity and on the amount of inoculum on the alternate host plants in the vicinity.

The Bouteloua grasses that develop within or on the margins of cotton fields commonly grow much larger and produce more infective telial material than desert-grown specimens, which are dependent on rainfall; hence, it is indicated that the practice of clean culture, including marginal areas, would be of value in control of the disease.

DIVISION OF COTTON AND OTHER FIBER CROPS AND
DISEASES, BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.

# DIPLODIA BLIGHT IN CONIFEROUS SEEDBEDS[1]

CHARLES M. SLAGG[2] AND ERNEST WRIGHT[3]

(Accepted for publication September 17, 1942)

While examining seedlings in a government nursery at Manhattan, Kansas, in August, 1941, the writers noted that first-year seedbeds of Douglas fir (*Pseudotsuga taxifolia* (Poir.) Britt.), Austrian pine (*Pinus nigra* Arnold), ponderosa pine (*P. ponderosa* Laws.), and piñon pine (*P. edulis* Engelm.) showed a number of dead and dying seedlings. The dark fruiting bodies of what appeared to be the causal fungus were plentiful on the dried central needles of the affected plants. Seedbeds of loblolly pine (*P. taeda* L.), slash pine (*P. caribaea* Morelet), and several small plantings of ponderosa pine from a California seed source were not affected.

This blight at first seemed to be similar to Phomopsis blight, which was under observation and treatment on adjoining eastern red-cedar seedbeds. Microscopic examinations on August 26 disclosed complete absence of *Phomopsis* and the presence of a single species of *Diplodia*. During the succeeding weeks the disease assumed serious proportions, and, by September 20, approximately 50 per cent of the seedlings of Douglas fir and Austrian pine were either diseased or dead. Injury to ponderosa and piñon pines was not so severe.

On September 25, an inspection was made of 3-year-old plantings of Austrian and Scotch pine (*P. sylvestris* L.) and of 5-year-old plantings of ponderosa and jack pine (*P. banksiana* Lamb.). A number of dead trees of Austrian and Scotch pines were found bearing the pycnidia and spores of the same *Diplodia* species found on the first-year seedlings. No first-year seedlings of Scotch pine were grown at the nursery in 1941, but infection of 3-year-old plantings was severe.

On October 9, a 50-year-old Austrian pine on the campus of Kansas State College at Manhattan was examined. About 50 per cent of the needles on the tree were dead and brown, and probably a much larger number had already fallen.

Numerous dark fruiting bodies were found on the basal portions of dead needles. Microscopic examination of the fruiting bodies disclosed the same species of *Diplodia* as that present at the government nursery 5 miles distant.

During the fall and winter of 1941–42 the fungus has been repeatedly found on mature trees of pitch (*P. rigida* Mill.), Table-Mountain (*P. pungens* Michx.), Austrian, Scotch, and ponderosa pines growing on the campus of Kansas State College. The most noticeable effect on these trees is a tip-

[1] Cooperative paper with the Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, and Kansas State College, Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station, contribution No. 425.

[2] Special Agent, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] Associate Pathologist, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

blight and dieback of the foliage. In the trees examined this blight first appeared in the youngest apical needle clusters, later progressing back along the stem until the entire shoot sometimes became involved.

The youngest apical needle clusters first formed the typical fruiting bodies of the fungus. During the autumn months, many dead shoots showed pycnidia only on these apical needles. Later, pycnidia were found on older needles, on the bark, and on the scales of the cones.

The fungus causing this dieback of mature trees and death of young seedlings is very similar to that discussed previously on conifers by various workers (3, 5, 7, 9, 10) as a *Sphaeropsis,* by others (1, 2, 6, 8) as a *Diplodia,* and by Curtis (4) as *Botryodiplodia.* In microscopic examinations of naturally infected specimens at Manhattan, from 0 to 87 per cent of septate pycnospores were observed in different mounts. It was noted that the septate spores were darker, and wider in proportion to their length, than the nonseptate spores. One hundred unicellular spores averaged 13.0 × 35.3 μ,

TABLE 1.—*Septation of Diplodia pinea as influenced by location of pycnidia on Scotch pine (Pinus sylvestris)*

| Location of pycnidia | Number of spores examined | Number septate | Number nonseptate | Percentage septate | Percentage pluriseptate |
|---|---|---|---|---|---|
| Long central needle | 1817 | 5 | 1812 | 0.27 | 0.0 |
| Short apical needle | 400 | 21 | 379 | 5.3 | 0.3 |
| Sheath at base of apical needle ..... | 300 | 153 | 147 | 51.0 | 1.0 |
| On bark of twig ..... | 200 | 132 | 68 | 66.0 | 1.5 |
| On scale of cone on same branch ..... | 281 | 243 | 38 | 86.5 | 2.5 |

while 100 septate spores averaged 14.65 × 32.1 μ. In crushed material the septate spores settled to the lower levels of water mounts and many were seen broken squarely across laterally, indicating the presence of a septum even though it might not be visible. Although there were wide variations in different pycnidia, about 25 per cent of the pycnospores observed were septate. The highest proportions of septate spores were found in mature overwintered pycnidia from large trees collected in March, 1942. Pluriseptate (usually 3-septate) spores were also fairly common in this material. Since fully ripened pycnidia always contained a higher proportion of septate spores than younger fruiting bodies, it seemed logical to assume that the bicellular spore was the mature form, and the fungus was identified as *Diplodia pinea* (Desm.) Kickx.

The causes for variation in septation of the pycnospores of *Diplodia pinea* are unknown. In the seedbeds, pycnidia on dead seedlings of Douglas fir produced only an occasional septate spore, while septation was abundant in pycnospores from adjacent similarly diseased seedlings of Austrian pine. Septation of pycnospores was always more pronounced in pycnidia formed near the bases of the pine needles than in pycnidia on other parts of the

needle. Table 1 shows the variation in septation of spores from pycnidia formed in different parts of a dead branch from a mature Scotch pine examined March 23, 1942.

Single-spore cultures of the fungus were readily secured from typical fruiting bodies on needles of first-year seedlings of Austrian pine. Three 1-foot-square blocks of uninfected Austrian pine seedlings, carefully removed with soil and roots undisturbed, were brought from a government nursery at Hutchinson to Manhattan, Kansas. A 15-day quarantine period was used to establish the absence of the disease and, on October 17, two of the three blocks of seedlings were inoculated and one was left as an uninoculated control. After inoculation all three lots were placed in a large damp chamber and left there for 72 hours. Upon removal they were placed on a greenhouse bench and examined daily.

Inoculation was accomplished in one block of seedlings by placing infected sporulating material from the nursery among the young plants. For some reason no infection resulted in this case. The other block of seedlings was inoculated with bits of young, vigorous mycelium from agar cultures placed on the uninjured growing points of all the plants in a section 6 inches square in the center of the flat. The mycelium rapidly invaded the tissues of the apical-needle cluster wherever applied and these apical needle clusters became discolored the fourth day after inoculation. By the fifth day, infection was apparent. On the tenth day the upper half of all inoculated seedlings was slate-colored and nearly dead. That portion of the block of seedlings that had been inoculated was smaller and the discoloration was marked as compared to the noninoculated seedlings in the same block, and also as compared to the control block, which remained vigorous and uninfected. Fifteen days after inoculation, a number of dark fruiting bodies were seen on the dead needles of inoculated seedlings. One such fruiting body was sectioned and examined under the microscope. It was a typical young pycnidium of *Diplodia pinea*. Thirty days after inoculation, all infected seedlings were dead.

The seedbeds had been sown approximately 5 months at the time the epiphytotic first became apparent. The Austrian pine seedlings that became diseased in the nursery and those used for inoculation purposes in the greenhouse were of substantially the same age and size, although grown in different nurseries, and had not yet completed development of woody tissues. At this stage of growth, infection rapidly became systemic and death of infected seedlings often occurred within a few days. It is important to note that infection in the cases here reported appeared to start first in the uppermost or youngest parts of the seedlings, also in the young centers of the needle clusters in the infected mature trees examined. In this respect the attack was quite different from that reported by Crandall (3) in his description of a root and collar disease of pine seedlings caused by *Sphaeropsis ellisii*.

A system of overhead watering in use at the nursery kept the seedbeds wet for a large part of the time. This fact, coupled with the splashing of

falling water, probably created favorable conditions for the spread of Diplodia infection after it became established in the beds.

There seems little doubt that this fungus has been parasitic upon various pines at Manhattan, Kansas, for many years. As far as the writers are aware, however, this is the first report of *Diplodia pinea* as a serious parasite of first-year seedlings.

SUMMARY

First-year seedlings of *Pinus nigra, P. edulis, P. ponderosa,* and *Pseudotsuga taxifolia* in a government nursery at Manhattan, Kansas, were severely blighted by a fungus identified as *Diplodia pinea* (Desm.) Kickx.

Uninjured seedlings of *Pinus nigra* were completely killed in 30 days by mycelial inoculations with a single-spore culture of *Diplodia pinea.* Typical pycnidia were formed on these dead seedlings 15 days after inoculation.

*Diplodia pinea* has been found on needles, needle bases, bark, and cone scales of "dieback" branches of mature trees of *Pinus nigra, P. ponderosa, P. pungens, P. rigida,* and *P. sylvestris* on the campus of Kansas State College at Manhattan, Kansas.

Septation of pycnospores of *Diplodia pinea* varied widely on different hosts and also varied on different plant parts.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
LINCOLN, NEBRASKA

LITERATURE CITED

1. BIRCH, T. T. C. *Diplodia pinea* in New Zealand. New Zeal. St. For. Serv. Bull. 8: 1–19. 1936.
2. BOYCE, J. S. Forest Pathology. 339 pp. McGraw-Hill Book Co. Inc. (New York). 1939.
3. CRANDALL, B. S. A root and collar disease of pine seedlings caused by *Sphaeropsis ellisii.* Phytopath. 28: 227–229. 1938.
4. CURTIS, K. M. A die-back of *Pinus radiata* and *Pinus muricata* caused by the fungus *Botryodiplodia pinea* (Desm.) Petr. New Zeal. Inst. Trans. & Proc. 55: 52–57. 1926.
5. HEDGCOCK, G. G. Notes on the distribution of some fungi associated with diseases of conifers. Pl. Dis. Rptr. 16: 28–42 (Mimeo.). 1932.
6. LUDBROOK, W. V., AND N. H. WHITE. Observations and experiments on Diplodia dieback of pines at Canberra. A. C. T. Australia Counc. Sci. Industr. Res. Journ. 13(3): 191–194. 1940.
7. PETRI, L. Dissecammento dei rametti di *Pseudotsuga douglasii* Carr. prodotto do una varieta di *Sphaeropsis ellisii* Sacc. Ann. Mycol. 11: 278–280. 1913.
8. SCHWARZE, C. A. The parasitic fungi of New Jersey. N. J. Agr. Exp. Stat. Bull. 313. 1917.
9. WATERMAN, ALMA M., and J. A. MILLER. A die-back of Douglas fir. Phytopath. 26: 804–805. 1936.

# CONVEX GUM, A NEW DISEASE OF CITRUS IN CHINA

LIN KUNG-HSIANG

(Accepted for publication September 10, 1942)

In the fall of 1941, a citrus-disease survey was made in Fukien Province, China. In an orange nursery at Kushanchow, Foochow, twig-blighting of a great majority of the nursery trees was strikingly apparent. These trees were about 4 years of age. When closely observed, the bark of the blighted twigs was seen to have many swellings with gum underneath, and, later, when the gum broke through, there were formed open irregular lesions, some of which girdled the twig in one or more places. In the open, broken lesions could be seen some dried gum. In an orchard at Chongha village, about 10 miles from Foochow City, a young tree was observed to have numerous swellings or convex areas on the bark of the trunk and limbs, but still lacked the later open lesions. When a convex area was cut open, a large quantity of gum was found in a pocket within the wood tissue. The disease was at once suspected to be the same as the more advanced destructive malady previously found on the orange nursery trees at Kushanchow. This conjecture was later confirmed. Observations were made of other orchards in the vicinity of Foochow City. Almost without exception, wherever orange trees were grown, the disease occurred.

## PLANTS AFFECTED

In Foochow, only sweet orange (*Citrus sinensis,* variety, Kushanchow Kan) was found affected, Foochow tangerine (*C. nobilis*) and Ponkan (*C. nobilis*) being free from the disease. The orange trees were commonly affected at a young stage. Only 2 older trees, about 30 years of age, were found to be affected.

## SYMPTOMS

The early symptoms of the disease were bark swelling and gum formation, which have suggested the name "convex gum," in contrast to the concave gum disease occurring in California, U. S. A. (2). There appear to be two types of the disease, so far as the symptom complex is concerned.

### Type 1

This type was by far the more common and the more serious of the two. Large, smooth, and somewhat round bark swellings appeared on the trunk, limbs, and twigs of the trees. The swellings were often numerous, and generally larger on the trunk than on the twigs, averaging about 2.5 cm. in diameter.

When one of the swellings was cut into, a large quantity of brownish, semiliquid-to-cheesy gum was found in a large flattened pocket in the wood, 1 to 5 mm. beneath the cambium layer. The gum extended some distance beyond the elevated area. The cambium, however, appeared unaffected.

No alternate layers of gum formation were apparent, as in the case of the concave gum disease (2).

After a large quantity of gum had accumulated in the pocket, it broke through the bark and formed a cankerlike lesion, which appeared much like the injury done by the larva of a twig borer (Fig. 1). Two or more such lesions often coalesced. When they occurred on the twig, the latter usually was girdled and blighted. With lesions on older trees, however, callusing and healing-over generally took place, and no serious damage to the tree was noticeable.

The foliage of the affected trees appeared normal, except on the young trees with blighted twigs, in which case yellowing occurred. Symptoms on leaves, characteristic of psorosis (1), were not found. Such symptoms may possibly appear on the young leaves, however, with the coming of the grow-

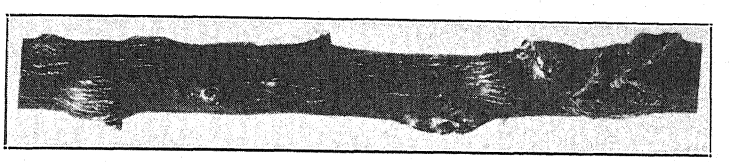


FIG. 1. A twig of *Citrus sinensis* affected with convex gum disease (type 1), showing bark swellings and cankerlike lesions.

ing season, as the disease is regarded to be a type of psorosis (see discussion under "Etiology").

### Type 2

This type was less common than type 1 and apparently caused no serious damage to the tree. Bark swellings on the trunk, limbs, and twigs of the trees were smaller than those of type 1, averaging about 1 cm. in diameter. The bark, when cut into, was found impregnated with gum, which appeared as numerous small, dark-brown spots.

## ETIOLOGY

While the bark swellings had escaped the notice of all the growers, the irregular, open lesions and the twig blighting had been seen and erroneously ascribed to an unknown insect injury.

Inasmuch as the wood and bark tissue covering the gum pocket were uninjured and appeared normal, indicating no possible path for the invasion by an organism, and in the light of the findings of Fawcett (1, 4) with respect to the etiology of psorosis, it is believed that the present disease is caused by a virus, possibly a strain of the psorosis virus. It is suspected that the disease may be in some way related to scaly bark, which is very common on the tangerine orange in both the Foochow and the Chiangchow districts of Fukien Province. The possibility that the trouble may be due to a minor-element deficiency should also be considered. Further investigations will be made as soon as conditions permit.

## EPIPHYTOLOGY

The disease is believed to be seed-transmitted; an assumption borne out by the fact that no trees propagated by the Chinese air-layering method, with the propagating limbs taken from healthy mother trees, have been found affected, while the diseased trees were always seedling trees.[1]

Evidence of the effect of phenological factors on the severity of the disease is not available. Observations so far made seem to indicate that convex gum may be aggravated by the lack of fertilizer, which supposedly weakens the tree.

## ECONOMIC IMPORTANCE

In the nurseries and young orchards, affected trees under the age of 5 years were either dying or badly stunted, while the older diseased trees might appear normal. The percentage of trees affected was generally high. In one nursery the incidence was 100 per cent, and the majority of the trees were dying. Counts were made of affected trees in several orchards in Foochow. The results are presented in table 1.

TABLE 1.—*The severity of convex gum of citrus in several orchards in Foochow*

| Orchard No. | Age of tree | Trees examined | Trees affected | Trees affected |
|---|---|---|---|---|
| | *Years* | *Number* | *Number* | *Per cent* |
| 1 | 4 | 24 | 24 | 100 |
| 2 | 9 | 31 | 11 | 35 |
| 3 | 9 | 10 | 8 | 80 |
| 4 | 7 | 14 | 6 | 43 |
| 5 | 7 | 25 | 16 | 64 |

From this table it may be seen that the percentage of infection ranged from 35 to 100. The average percentage was about 64. Damage resulting from the disease may not be limited to the killing and stunting of the young trees. Affected trees that have temporarily recovered and appear normal may deteriorate sooner than the healthy trees, as is the case with psorosis-affected trees in the United States. It is obvious that the disease inflicts heavy losses to both the nursery men and the growers.

## CONTROL

Since the disease appears to be seed-transmitted, it is suggested that mother trees be selected and placed under observation for 2 to 3 years and, if found free from the disease, be registered as certified trees for propagation, as is being done for the control of psorosis in California (3). Remedial measures are suggested by the experience of a grower at Kushanchow. A seriously affected tree, about 30 years old, recovered after the excision of affected tissue and application to the soil of a generous quantity of fertilizer. The lesions had all healed over, and the tree had regained a normal, healthy appearance. Such a method is in accord with what has sometimes been

[1] The great majority of oranges in Foochow are propagated by seedling trees.

employed by orange growers in California for the treatment of slight or medium cases of concave gum (2).

SUMMARY

A new citrus disease, called "convex gum," has been found in Southern China. The disease is characterized by bark swelling and gum formation on the trunk, limbs, and twigs, and it appears to be of two types. In type 1 the bark swelling is larger than in type 2, and a large quantity of gum is produced in a pocket in the wood tissue, 1 to 5 mm. underneath the cambium layer. The gum may break through the bark and form cankerlike lesions, which may coalesce, later girdling and killing the twigs. Young trees may be killed or badly stunted. In type 2, with smaller swellings, gum is formed in the bark and shows as small brown flecks. The disease is believed to be caused by a virus, probably related to the one causing citrus psorosis, or scaly bark.

CITRUS RESEARCH INSTITUTE,
LINGNAN UNIVERSITY,
PINGSHEK, KWANGTUNG, CHINA.

LITERATURE CITED

1. FAWCETT, H. S. Is psorosis of citrus a virus disease? Phytopath. 24: 659–668. 1934.
2. ————. Concave gum disease. *In:* Citrus diseases and their control. 2d ed. 656 p. (see specifically p. 215–216). McGraw-Hill Book Company, Inc. (New York and London). 1936.
3. ————. Disease-free parents for psorosis prevention. Pacific Rural Press 137 (12): 280. 1939.
4. ————. Citrus viruses. Phytopath. 31 (4): 356–357. 1941.

# THE EFFECT OF SOIL AND CHEMICAL MIXTURES ON THE GROWTH OF FUSARIUM OXYSPORUM CUBENSE[1]

CLIFFORD H. MEREDITH

(Accepted for publication July 25, 1942)

The rate of growth of *Fusarium oxysporum cubense* has been recorded in several soil types.[2] Observations have been made on the highest non-toxic percentage and the lowest toxic percentage of chemical and soil-culture mixtures.[3] The work here reported has been an effort to find out the ability of chemicals to prevent the growth of the fungus in the soil.

Air-dried clay soil was used in the experiment. The moisture was determined, and the percentages given are those of the chemical ingredient and

TABLE 1.—*Chemicals that have not been observed to influence the growth of Fusarium oxysporum cubense in 1 per cent soil mixtures*

| | | |
|---|---|---|
| Aluminum sulphate | Citric acid | Potassium chromic sulphate |
| Ammonium chloride | Cobaltous nitrate | Potassium permanganate |
| Ammonium chromic sulphate | Cupric sulphate | Potassium sodium tartrate |
| Ammonium molybdate | Gas oil | Potassium sulphate |
| Ammonium nitrate | Hydrate line | Sodium carbonate |
| Ammonium sulphate | Kerosene oil | Sodium carbonate, acid |
| Antimonic acid | Lead arsenate | Sodium chloride |
| Barium bromide | Lead oxalate | Sodium hydroxide |
| Barium carbonate | Lead oxide, mono- | Sodium nitrate |
| Barium chloride | Lead oxide, red | Sodium nitrite |
| Barium nitrate | Magnesium chloride | Sodium tetrasilicate |
| Barium oxide | Manganous sulphate | Stannous chloride |
| Barium peroxide | Mercuric sulphide | Stannic oxide |
| Benzidine | Oxalic acid | Strontium carbonate |
| Bismuth oxycarbonate | Potassium aluminum sulphate | Strontium nitrate |
| Bismuth oxynitrate | Potassium antimony tartrate | Sugar |
| Calcium chloride | Potassium bromide | Zinc oxide |
| Calcium fluoride | Potassium chloride | Zinc sulphate |
| Calcium oxalate | | |

of the soil dried at 100° C. until a constant weight was obtained. The chemical was added with water up to 3 ml. to test tubes containing 10 g. of soil. The tubes were autoclaved and planted with *Fusarium oxysporum cubense*. The growth of the fungus was measured 7 days and 14 days, respectively, after planting.

There were 21 chemicals that inhibited the growth of the fungus in concentrations of 1 part per 100 or less (Table 2). Of the inhibiting chemicals there were 9 that prevented the growth at 1 part per 100 only, 1 chemical at 1 part per 1000, 8 chemicals at 1 part per 10,000, and 3 chemicals at 1 part per 100,000. Of the chemicals under observation 16 decreased the rate of growth of the organism but did not prevent the growth at 1 part per 100

[1] Acknowledgment is due the Jamaica Banana Producers' Association for support of this research.

[2] Meredith, C. H. The growth of *Fusarium oxysporum cubense* in the soil. Phytopath. 31: 91–93. 1941.

[3] Meredith, C. H. The effect of chemicals on *Fusarium oxysporum cubense* growing in the soil. Phytopath. 32: 182–184. 1942.

concentration. Copper oxalate, ferric sulphate, hydrochloric acid, lead acetate, magnesium sulphate, nickel sulphate, nitric acid, potassium bromide,

TABLE 2.—*The penetration of Fusarium oxysporum cubense in chemical and soil mixtures*

| Chemical | No. of days | One part per 100 | One part per 1,000 | One part per 10,000 | One part per 100,000 | One part per 1,000,000 | Check | Check |
|---|---|---|---|---|---|---|---|---|
| | | *cm.* | *cm.* | *cm.* | *cm.* | *cm.* | *cm.* | *cm.* |
| 35. Acetic acid | 7 | ..... | ..... | 1.6 | 1.4 | 1.5 | 1.9 | 1.6 |
| | 14 | ..... | 0.1 | 3.4 | 3.6 | 3.2 | 3.1 | 3.0 |
| 70. Borax | 7 | ..... | 1.2 | 1.5 | 1.2 | 1.6 | 1.5 | 1.6 |
| | 14 | ..... | 2.5 | 2.5 | 2.3 | 2.7 | 2.3 | 2.2 |
| 15. Boric acid | 7 | ..... | 0.5 | 1.1 | 0.9 | 1.6 | 1.9 | 1.3 |
| | 14 | ..... | 0.5 | 2.2 | 3.0 | 2.6 | 3.2 | 2.1 |
| 100. Cadminum sulphate | 7 | ..... | 1.1 | 1.5 | 1.5 | 1.9 | 1.5 | 1.8 |
| | 14 | ..... | 2.3 | 3.6 | 3.0 | 7.5 | 3.3 | 3.7 |
| 76. Iodine | 7 | ..... | ..... | 0.5 | 0.7 | 1.0 | 1.3 | 1.5 |
| | 14 | ..... | ..... | 0.5 | 0.8 | 3.7 | 2.4 | 3.2 |
| 87. Mercuric ammonium chloride | 7 | ..... | ..... | ..... | 0.5 | 1.2 | 1.5 | 1.6 |
| | 14 | ..... | ..... | ..... | 0.5 | 2.5 | 3.5 | 3.5 |
| 89. Mercuric carbonate | 7 | ..... | ..... | ..... | 0.3 | 0.5 | 1.4 | 1.1 |
| | 14 | ..... | ..... | ..... | 0.2 | 0.5 | 3.0 | 3.5 |
| 7. Mercuric chloride | 7 | ..... | ..... | ..... | ..... | 0.7 | 1.0 | 1.8 |
| | 14 | ..... | ..... | ..... | ..... | 2.1 | 2.4 | 2.6 |
| 88. Mercuric iodide | 7 | ..... | ..... | ..... | ..... | 0.6 | 1.3 | 1.4 |
| | 14 | ..... | ..... | ..... | ..... | 2.8 | 2.6 | 3.1 |
| 53. Mercuric oxide (red) | 7 | ..... | ..... | ..... | 0.1 | 1.0 | 1.3 | 1.5 |
| | 14 | ..... | ..... | ..... | 0.1 | 1.8 | 3.0 | 3.3 |
| 54. Mercuric oxide (yellow) | 7 | ..... | ..... | ..... | ..... | 0.2 | 1.9 | 1.2 |
| | 14 | ..... | ..... | ..... | ..... | 0.3 | 3.0 | 3.1 |
| 90. Mercuric sulphate | 7 | ..... | ..... | ..... | 0.8 | 1.5 | 1.8 | 1.6 |
| | 14 | ..... | ..... | ..... | ..... | 2.5 | 3.0 | 3.2 |
| 16. Mercurous chloride | 7 | ..... | ..... | ..... | 0.8 | 1.1 | 1.5 | 1.8 |
| | 14 | ..... | ..... | ..... | 2.3 | 2.8 | 4.9 | 3.0 |
| 3. Mercury dust No. 1 | 7 | ..... | ..... | ..... | 0.4 | 1.3 | 1.4 | 1.9 |
| | 14 | ..... | ..... | ..... | 0.3 | 3.2 | 3.1 | 3.0 |
| 4. Mercury dust No. 2 | 7 | ..... | ..... | ..... | 0.4 | 0.5 | 2.1 | 1.7 |
| | 14 | ..... | ..... | ..... | 2.3 | 2.5 | 2.9 | 3.3 |
| 60. Mercury dust No. 3 | 7 | ..... | ..... | ..... | 0.2 | 0.7 | 1.2 | 2.4 |
| | 14 | ..... | ..... | ..... | 0.2 | 0.5 | 2.5 | 4.0 |
| 48. Paris green | 7 | ..... | 1.5 | 1.5 | 1.0 | 1.7 | 1.7 | 1.2 |
| | 14 | ..... | 3.5 | 3.1 | 2.7 | 3.7 | 3.3 | 2.5 |
| 45. Phenolphthalein | 7 | ..... | 1.4 | 1.4 | 1.7 | 1.5 | 1.2 | 1.5 |
| | 14 | ..... | 3.4 | 3.8 | 3.5 | 3.0 | 3.4 | 3.2 |
| 2. Potassium dichromate | 7 | ..... | 0.9 | 1.4 | 1.7 | 1.5 | 1.5 | 1.9 |
| | 14 | ..... | 2.3 | 2.9 | 3.2 | 2.7 | 3.4 | 3.0 |
| 8. Silver nitrate | 7 | ..... | 0.2 | 1.5 | 1.5 | 1.4 | 1.3 | 1.3 |
| | 14 | ..... | 0.2 | 2.2 | 2.6 | 2.0 | 2.6 | 1.7 |
| 42. Thymol | 7 | ..... | 1.2 | 1.0 | 1.2 | 1.0 | 1.1 | 1.4 |
| | 14 | | 2.6 | 2.3 | 2.9 | 2.8 | 3.5 | 2.8 |

potassium chlorate, sulfanilamide, sulphur, sulphuric acid, and 4 commercial sprays decreased the growth. There were 8 phosphorus compounds used in this study. Calcium phosphate, di-sodium phosphate, sodium am-

monium phosphate, sodium biphosphate, sodium di-hydrogen phosphate, sodium phosphate, superphosphate, and tri-sodium phosphate gave greater growth in concentrations of 1 part per 100 and 1 part per 1000 than the soil without the phosphorus in the check tubes. In this experiment 55 chemicals were not observed to affect the growth of the Panama disease organism in a 1 per cent mixture (Table 1).

The mercury compounds were the most effective in preventing the growth of *Fusarium oxysporum cubense* in the soil. Of the 12 mercury compounds observed 11 were able to inhibit the growth of the organism in concentrations of less than 1 part per 10,000.

GLENLEIGH LABORATORY, FRIENDS COLLEGE,
HIGHGATE, JAMAICA, B. W. I.

# THE COMPOSITION OF WHITE CLOVER LEAVES AS AFFECTED BY RUST AND BY SULPHUR[1]

J. T. SULLIVAN AND S. J. P. CHILTON

(Accepted for publication September 4, 1942)

A previous communication[2] reported the influence of leaf rust (*Uromyces trifolia* (Hedw. f.) Lév.) and of sulphur, that was used to control the rust, in decreasing the carotene content of white clover (*Trifolium repens* L.). Analyses for other constituents indicate that sulphur and rust exert an influence on some of these constituents. As no further analyses can be made in the near future, the results, though preliminary, are reported.

The plants used were the same as in previous studies.[2] Comparative analyses of rusted and sulphur-dusted susceptible plants are given in table 1. Moisture is expressed on the fresh-weight basis, other constituents on the

TABLE 1.—*Comparative analyses of rusted and rust-free plants of white clover*

| Constituent materials | Average of 7 susceptible clones | | | Average of 2 resistant clones | | |
|---|---|---|---|---|---|---|
| | Dusted | Rusted | F value | Dusted | Not dusted | F value |
| | *Per cent* | *Per cent* | | *Per cent* | *Per cent* | |
| Moisture | 77.20 | 75.87 | 10.57[a] | 79.76 | 80.01 | 0.17 |
| Crude protein N | 3.22 | 3.36 | 7.17[b] | 3.59 | 3.87 | 26.41[c] |
| Ash | 14.37 | 15.08 | 4.06 | 13.40 | 12.84 | 17.15[c] |
| Fat | 1.88 | 2.37 | 55.23[a] | 2.08 | 2.28 | 2.40 |
| Fiber | 13.79 | 13.49 | 2.35 | 12.58 | 11.98 | 29.42[c] |
| N-free extract | 49.76 | 48.01 | 10.59[a] | 49.28 | 48.54 | 1.69 |

[a] Exceeds 1% point of 7.72.
[b] Exceeds 5% point of 4.22.
[c] Exceeds 1% point of 13.74.

dry-weight basis. From the data it is obvious that rusted plants were higher in protein and fat and lower in moisture and nitrogen-free extract than were sulphured plants. Analyses of dusted and nondusted, rust-resistant plants indicate (Table 1) sulphur has increased the ash and fiber and decreased the protein.

It is assumed that sulphur dusting affected rust-susceptible and rust-resistant plants equally. Therefore, to evaluate properly the effect of rust on susceptible plants, the effect of sulphur on the sulphured plants with which they are compared should be taken into account. Since sulphur has decreased the protein, the observed effect of rust in causing an increase in protein cannot be verified. The ash content has been increased by the rust to a greater degree than the data indicate and the difference is probably of

[1] Contribution No. 40, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Northeastern States.

[2] Sullivan, J. T., and S. J. P. Chilton. The effect of leaf rust on the carotene content of white clover. Phytopath. 31: 554–557. 1941.

significance. The effects of sulphur on the fat and on the nitrogen-free extract, while not of statistical significance, are in such a direction as to bring into question the significance of the effects of rust on the susceptible plants.

Another approach to the problem of determining the effect of rust is to compare the composition of rusted with non-rusted leaves from the same plant. Such a comparison is not complicated by the effect of sulphur. Leaves from rusted plants were grouped by hand as heavily rusted, lightly rusted and non-rusted. Analyses of these samples are summarized in table 2. The differences found here between rusted and non-rusted leaves are greater than those illustrated in table 1, but the rusted samples described in table 1 were of all leaves taken from rusted plants and not all leaves of such plants were rusted.

The rusted leaves are higher in ash, fat, fiber, and nitrogen-free extract and lower in moisture and protein than non-rusted leaves of the same plant (Table 2). All differences are significant. Though only fully developed

TABLE 2.—*Comparative analyses of leaves from same plant (average of 9 plants)*

| Constituent materials | Heavily rusted leaves | Lightly rusted leaves | Non-rusted leaves | F value |
|---|---|---|---|---|
| | *Per cent* | *Per cent* | *Per cent* | |
| Moisture | 70.11 | 72.74 | 76.23 | 85.89[a] |
| Crude protein N | 3.21 | 3.47 | 4.03 | 167.63[a] |
| Ash | 15.28 | 14.38 | 14.10 | 7.74[a] |
| Fat | 2.56 | 2.21 | 1.99 | 38.44[a] |
| Fiber | 13.61 | 12.75 | 12.49 | 32.35[a] |
| N-free extract | 48.54 | 48.96 | 46.24 | 18.19[a] |

[a] Exceeds 1% point of 6.23.

leaves were used, the composition of the non-rusted leaves as compared with the rusted is similar to that of younger as compared with older leaves. While more younger leaves may have been included in the non-rusted group, the differences in composition found are not believed wholly attributable to age. Ash content, the only constituent definitely shown increased by rust, serves as a criterion. The difference in ash content between heavily rusted and non-rusted leaves are what would be expected from the effects of rust alone. In another experiment leaves from the older halves of stolons of 3 rust-free clones averaged 16.3 per cent more ash than did leaves from the younger halves of the same stolons. If manual separation of rusted and non-rusted leaves placed a greater proportion of young leaves in the rust-free class the differences in ash caused by the two factors of age and rust should have been greater than that found.

It is concluded that both rust and sulphur have affected the composition of white clover plants and that sulphured plants cannot be used as controls in determining the effects of rust on certain chemical constituents of the plant.

## PHYTOPATHOLOGICAL NOTES

*The Antagonism of Actinomyces to Fusarium oxysporum cubense.*[1]—A species of *Actinomyces* has been isolated that will dissolve the mycelium of *Fusarium oxysporum cubense.* The culture was collected from a compost heap made by Clifford L. Clemetson at Frontier, Port Maria, P. O., Jamaica. The agent that produced the lysis diffused through agar and checked the growth of the fungus. When spores of each organism were mixed together in a Petri dish of 2 per cent soil-extract agar the fusarium apparently developed for two days in a normal manner when compared with the check plates. The lysis was very evident on the fifth day, for sections of the mycelium had disappeared with small sections remaining. Some *Fusarium* chlamydospores were observed on the seventh day. In 9 days the fungus had disappeared and the *Actinomyces* sp. had made a normal growth similar to the check plates.—CLIFFORD H. MEREDITH, Glenleigh Laboratory, Highgate, Jamaica, B.W.I.

*Resistance in the Genus Nicotiana to Phytophthora parasitica Dastur var. nicotianae Tucker.*—Thirteen *Nicotiana* species, including several strains and varieties of *N. tabacum,* have been tested, under greenhouse conditions, for resistance to black shank. All plants were transplanted to steam-sterilized soil previously inoculated with pure cultures of *Phytophthora;* all cultures having been isolated from infected tobacco and tested for pathogenicity. Data were taken as to total number of plants killed or partially infected; roots of surviving plants were examined for infection.

Under the severe conditions of these experiments no *Nicotiana* species or strains proved immune. Two varieties or strains of *N. tabacum,* Florida Rg and Tobacco Institute 150 (53–A), showed some resistance provided plants, set in inoculated soil, were approximately of size and age for field transplanting. When small seedlings of these varieties were set in inoculated soil nearly all plants were infected and a high percentage killed. Numerous observations of these varieties, grown in outside beds in naturally infested soil, give added support to data from these experiments, indicating that Rg and 150 (53–A) show but little, if any, resistance to black shank in the early seedling stage, under Puerto Rican conditions. Two commercial varieties, Virginia No. 9 and Utuado X No. 1, have shown some degree of resistance under field conditions based on commercial field observations and confirmed by data from a randomized field-plot experiment.[1] However, in these greenhouse experiments nearly all plants of Virginia No. 9 and Utuado X No. 1 were killed.

Three additional *Nicotiana* species, *N. repanda, N. rustica,* and *N. longiflora,* showed definite resistance to black shank. In *N. repanda* resistance

[1] Acknowledgment is due the Jamaica Banana Producers' Association for support in this research.

[1] Foster, H. H., M. García Fortuño and G. Irizarry Rubio. Notes on diseases, decays, and disorders of tobacco in Puerto Rico during the 1941–42 season. (Mimeographed) Pl. Dis. Rptr. **26**: 247–253. 1942.

appeared to be located primarily in the roots. All infected plants observed showed active infection to be limited to the more succulent stems and leaves. In several plants when the disease reached the transition zone between stem and roots, infection was "corked" off. Plants of sufficient size and vigor to continue stem and leaf production appeared to recover completely. From the results of these experiments *N. repanda* might be of definite value in inter-species crosses with *N. tabacum*. It has been shown[2, 3] that *N. repanda* carries resistance to several other major tobacco diseases. Earlier, Tisdale[4] found that *Nicotiana rustica* was resistant to black shank but failed, in all attempts, to cross *N. rustica* with *N. tabacum*.

Numerous attempts were made, by the writer, to cross *Nicotiana repanda* with *N. tabacum*, using several different combinations. Germination of $F_1$ seed was obtained from a cross between (4–N *repanda*) × (2–N) *tabacum*. The (4–N) *repanda* plants were grown from colchicine-treated seed. All $F_1$ plants grew somewhat slowly, and, following transplanting, failed to recover and initiate new growth.—H. H. FOSTER, Department of Pathology and Genetics, Tobacco Institute of Puerto Rico, Rio Piedras, P. R.

*Leaf Spot on Terminalia arjuna.*—In February, 1937, while visiting the United States Plant Introduction Garden at Coconut Grove, Dade County, Florida, the writer observed a severe leaf spot on *Terminalia arjuna* Wight and Arn. growing in the garden. This conspicuous disease apparently has not been reported hitherto. It has continued abundant as shown by specimens received in subsequent years. These were collected by H. F. Loomis, who, with L. G. Polhamus, was present when the disease was discovered. The leaf spot appears on any part of the blade, and is circular to irregular and may be delimited by the veins. Usually, it is 2–8 mm. in diameter but is sometimes more extensive. Based on dry specimens, the color of the spot, above, is wood-brown, light brownish-drab, or pale-drab gray, and, below, mikado-brown to tawny-olive.[1] The necrotic area becomes dry, shrunken, and severed partly or entirely from the leaf as a whole. Severely infected leaves from which much of the dead tissue has fallen away present an extremely ragged or insect-eaten appearance. (Fig. 1, A–C.)

The disease seems to be of fungus origin. Pycnidia of the nature of *Phyllosticta* are present on the lesions, and among the organisms isolated is *Phomopsis* sp. (Fig. 1, D and E), as well as *Pestalotia*. The *Pestalotia* has been identified as *P. disseminata* Thüm. by E. T. Guba, who stated that this species, originally described on *Eucalyptus*, is widespread among members of the Myrtales.—ANNA E. JENKINS, Bureau of Plant Industry Station, Beltsville, Md.

[2] Clayton, E. E. Resistance to root-knot nematode in Nicotiana. (Abstract) Phytopath. 30: 708–709. 1940.

[3] Clayton, E. E., and H. H. Foster. Disease resistance in the genus Nicotiana. (Abstract) Phytopath. 30: 4. 1940.

[4] Tisdale, W. B. Development of cigar wrapper tobacco resistant to black shank. (*Phytophthora nicotiana* Breda de Haan) Florida Agr. Exp. Stat. Bull. 226, 1931.

[1] Color readings based on Ridgway, R. Color standards and color nomenclature. 43 pp. illus. Washington, D. C. 1912.

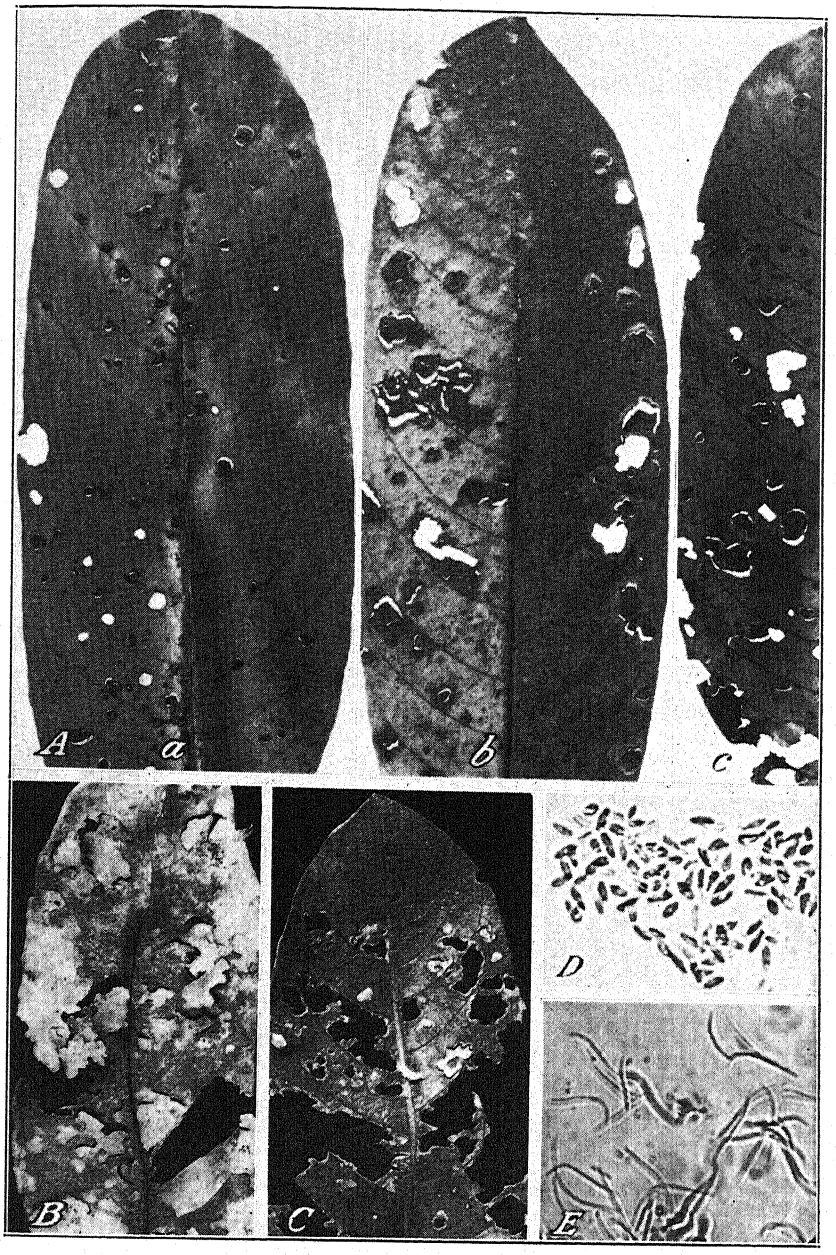



FIG. 1. A–C. Spotting of old leaves of *Terminalia arjuna*, U. S. Plant Introduction Garden, Coconut Grove, Fla. A, a and c, May, 1938, H. F. Loomis; A, b, February, 1937, L. G. Polhamus; B and C, January, 1939, H. F. Loomis. All × 1. D and E. *Phomopsis* sp., D, alpha spores; E, beta spores. × 500. All photographs by M. L. F. Foubert.

*A Differential Medium for the Isolation of Phytomonas sepedonica.*[1]—The study of the ring rot of potatoes frequently involves numerous routine isolations of the pathogen (*Phytomonas sepedonica*). This operation is relatively simple when the tubers employed are in the early stages of infection. If, however, the isolation is attempted from badly rotted tubers, sec-

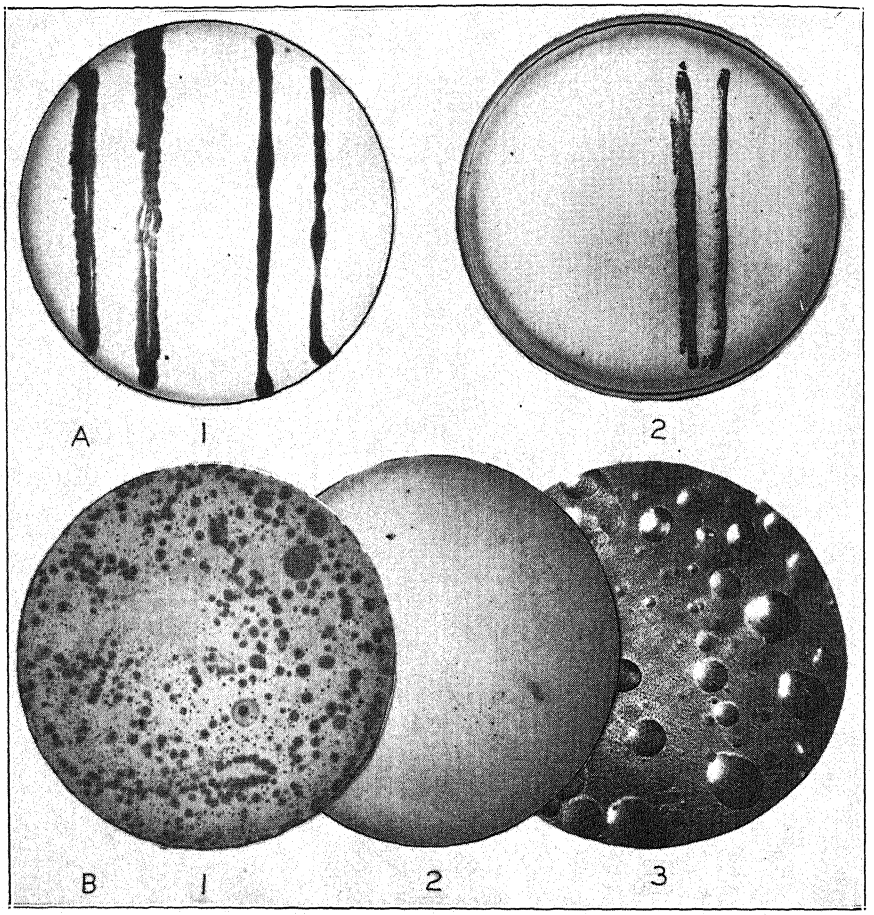



FIG. 1. Agar plate cultures showing effect of potassium dichromate on growth of soft-rot and ring-rot bacteria. A1. A control plate of Burkholder's agar streaked with a suspension of a pure culture of soft-rot bacteria (*Erwinia carotovora*) at left and *Phytomonas sepedonica* on right. A2. A plate of dichromate agar (1: 12,000) streaked with the two organisms. Note good growth of *P. sepedonica* and complete inhibition of *E. carotovora*. B1. A dilution plate of Burkholder's medium inoculated with mixture of soft-rot and ring-rot bacteria. Soft-rot colonies have overgrown the plate, completely obscuring any ring-rot colonies that might have developed. B2. A plate of dichromate agar (1: 12,000) inoculated in the same manner. The soft-rot bacteria were inhibited completely, while the ring-rot bacteria were present, although not visible here because of the small size of the colonies. B3. The colonies of *P. sepedonica* in the dichromate-agar plate shown in B2, photographed at 100 × magnification.

[1] Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 299.

ondary organisms of the soft-rot type (Gram-negative rods) usually crowd out the pathogen. A differential medium that largely overcomes this difficulty has been devised. It is based on Mallmann's[2] observation that potassium dichromate inhibits Gram-negative bacteria, while it permits the growth of Gram-positive species.

Burkholder's medium,[3] and a 1:1000 solution of C.P. potassium dichromate are the basic materials. These are prepared in separate flasks and sterilized. A heavy suspension of the yellowish exudate from the ring-rot lesion is made in sterile water. From this master suspension, loop dilutions are made to a series of duplicate plates. The liquefied agar, cooled to 50° C. is mixed with the calculated amounts of sterile 1:1000 potassium dichromate solution. Usually dilutions of 1:9000, 1:12,000, and 1:15,000 give satisfactory concentrations to accommodate the varying conditions incident to isolation. Approximately 10 ml. of the medium is poured over the bacterial suspension and thoroughly mixed. The cultures are incubated at 22° C. in a moist chamber. After 7 to 9 days, distinct pin-point colonies of the ring-rot bacteria appear in the agar. Isolation of these colonies is easily effected by the usual technique of "fishing," using a finely sharpened chromel wire needle for this purpose. The sharp differential action of this medium is shown in figure 1.—E. A. MARTEN, C. V. LOWTHER, and J. G. LEACH, Department of Plant Pathology and Bacteriology, West Virginia University, Morgantown, W. Va.

*Leaf Blight of Corn.*—Leaf blight of dent corn has been under observation in Ohio for the last 4 seasons. With the introduction and widespread use of corn hybrids it has become more important, especially in the southern half of the State. It is estimated that the loss resulting from leaf blight in southern Ohio in the last 4 years has exceeded that from any other disease. Work was initiated in 1939, in Ohio, to determine the major cause or causes of the disease. Many growers of corn hybrids were much concerned; the trouble was variously attributed to drought injury, poor soil conditions, and Stewart's disease or bacterial leaf blight.

During July, August, and September, 1939 more than 200 isolations were made from collections of diseased corn leaves from different regions in southern Ohio. Pathogenicity of the isolated organisms was tested. Bacterial isolates were hypodermically injected into stalks of 6- to 8-inch greenhouse-grown corn plants, while the isolated fungi were tested by spraying like plants with a spore suspension in water and then maintaining them 24 to 36 hours in a moist chamber. These experiments indicated two kinds of leaf blight in Ohio: bacterial, caused by *Phytomonas stewarti* (Sm.) Com. S. A. B., and Helminthosporium leaf blight caused by *Helminthosporium turcicum* Pass. The latter being the more prevalent.

[2] Mallmann, W. L. The selective bacteriostatic effect of slow oxidizing agents. Jour. Bact. 42: 295. 1941.

[3] Burkholder, W. H. The occurrence in the United States of the tuber ring rot and wilt of the potato. Amer. Potato Jour. 15: 243–245. 1938.

Symptoms characterizing the two blights differ distinctly and are usually readily distinguishable in the field. The bacterial lesions are spots of irregular shape, or streaks often extending almost the entire length of the leaf. The individual spots produced by *H. turcicum* are more or less elliptical, 1 to 4 cm. wide by 14 cm. or less long; often a brown to reddish margin, with zonation apparent in some spots. The center of the fungus lesions is often covered with a greenish-black growth of conidia and conidiophores. Coalescence of lesions may occur in both types of blight, and the leaf soon dies and becomes dry.

Preliminary tests of the reaction of inbred lines, single crosses, and double crosses of dent corn to *Helminthosporium turcicum* were made in the greenhouse. Plants of 24 inbred lines, 26 single crosses and 6 double crosses were sprayed with a water suspension of spores and then placed in a moist chamber for 24 to 36 hours. The results showed no seedling resistance in any of these lines or crosses.

The isolation and inoculation tests suggest that the fungus *Helminthosporium turcicum* induces most of the leaf blight in Ohio, a fact further established by many observations of experimental breeding plots, cooperative test plots, and farm fields made in August and September, 1940, 1941, and 1942. Marked differences in amount of infection also were then noted between the different inbreds and crosses grown side by side. Inbred lines KYS, Ia. L317, Ia. L289, Ohio 02 and Ohio 40B are some that are resistant to Helminthosporium blight, while Ia. 1224, Ill. A. U. S. 540, Wis. CC5 and Ohio 67 are very susceptible. Hybrids Ia. 939, U. S. 13 and Ohio L86 have been resistant, while Ind. 614, Ind. 608C, U. S. 44, and Ohio C14 are very susceptible in southern Ohio. In 1938 Ohio produced 316 acres of hybrid Indiana 614 seed. By 1942 there was only 11 acres, due chiefly to Helminthosporium leaf blight.

Although bacterial leaf blight has been present in many Ohio fields in the last four years, it has been observed causing serious losses in but few instances. Although present in many Ohio fields since 1939 it has been observed causing serious losses in but few instances.

*Helminthosporium turcicum* also has been isolated from Sudan-grass (*Sorghum vulgare* var. *sudanense* (Piper) Hitchc.) and Johnson-grass (*Sorghum halepense* (L.) Pers.) collected in southern Ohio.—C. W. ELLETT,[1] Department of Botany, The Ohio State University, Columbus, Ohio.

*Influence of Plant Populations upon Incidence of Pineapple Yellow Spot.*[1] —Recent studies on the influence of tomato plant populations and planting systems upon incidence and destructiveness of the curly-top disease,[2] add interest to data taken by the writer some years ago on yellow-spot of pine-

[1] Acknowledgment is made to Dr. C. C. Allison and Dr. W. G. Stover for helpful suggestions in the course of the work.

[1] Published with the approval of the Acting Director as Technical Paper No. 144 of the Pineapple Research Institute of Hawaii, University of Hawaii.

[2] Shapovalov, M., H. L. Blood, and R. M. Christiansen. Tomato plant populations in relation to curly-top control. (Abstract) Phytopath. 31: 864. 1941.

apple, *Ananas comosus* (L.) Merr. This disease is transmitted by *Thrips tabaci* Lind.,[3] and is caused by a virus probably identical with that of tomato spotted wilt.[4,5] Infection of pineapple plants results chiefly or exclusively from the feeding of thrips that have developed on more favorable hosts of both virus and vector. Pineapple plants are susceptible during early growth, soon after planting, and again during development of the fruit. Plants infected early die without fruiting; hence, this disease in fruiting plants represents infection after the inflorescence has begun to develop.

In a plantation experiment in which population density had been varied by 3 widths of spacing between plants in the rows of 4-row beds, the prev-

TABLE 1.—*Incidence of yellow-spot of pineapple fruits and crowns, per cent and per acre, related to 3 intervals of spacing of plants in the rows and the resulting numbers of plants per acre*

| Replication | Infections per 100 plants | | | Infections per acre | | |
|---|---|---|---|---|---|---|
| | 12 in. | 15 in. | 18 in. | 12 in. | 15 in. | 18 in. |
| | 21,780 | 18,150 | 14,520 | 21,780 | 18,150 | 14,520 |
| | *Per cent* | *Per cent* | *Per cent* | *No.* | *No.* | *No.* |
| 1 | 3.0 | 4.8 | 5.5 | 653 | 871 | 799 |
| 2 | 3.5 | 4.3 | 5.3 | 762 | 780 | 770 |
| 3 | 3.4 | 4.6 | 4.6 | 740 | 835 | 668 |
| 4 | 3.3 | ..... | 5.4 | 719 | ..... | 784 |
| Mean | 3.3 | 4.6 | 5.2 | 718 | 829 | 755 |
| Difference, per cent | ..... | 39.4 | 57.6 | ..... | 15.5 | 5.2 |
| F[a] | ..... | ..... | 67.7[b] | ..... | ..... | 0.94 |

[a] With 1 and 6 degrees of freedom in comparisons of 12 inch and 18 inch spacings, F = 5.99 for 5 per cent probability and F = 13.74 for 1 per cent.

[b] After angular transformation of percentages to degrees, the calculated F value is 72.5.

alence of yellow-spot infection was determined just prior to harvest. All beds consisted of 4 rows 17 inches apart, with the beds spaced 8 feet from center to center; but plants in the rows were spaced 12, 15, and 18 inches apart, resulting in populations of 21,780, 18,150, and 14,520 plants per acre, respectively, when the area occupied by field roads is not considered. Each spacing was represented by 4 replications of 4-bed plots 300 feet long, with plots and treatments arranged parallel in systematic rotation in a strip 384 feet wide. Fertilizer had been applied before planting at a uniform rate per unit area. Later applications were uniform per plant.

Counts were made of fruiting plants in each plot and of plants with fruits or crowns or both infected with yellow-spot. The data are presented

[3] Linford, M. B. Transmission of the pineapple yellow-spot virus by *Thrips tabaci*. Phytopath. 22: 301–324. 1932.

[4] Parris, G. K. Mechanical transmission of yellow-spot virus: evidence for identity with spotted-wilt virus. Phytopath. 30: 299–312. 1940.

[5] Sakimura, K. Evidence for the identity of the yellow-spot virus with the spotted-wilt virus: experiments with the vector, *Thrips tabaci*. Phytopath. 30: 281–299. 1940.

in table 1 on a percentage basis and as calculated numbers of infected plants per acre.

Consistency of the percentage of infection among plots of equal population density is sufficient to demonstrate the significance of differences between spacings. The 12- and 18-inch spacings are clearly distinct, with 57.6 per cent heavier infection in the latter. The unfortunately incomplete data from the intermediate spacing overlap somewhat those from the widest spacing, but indicate an intermediate percentage of infection.

Numbers of infections per acre, on the contrary, show no such significant influence of plant population. The apparent differences between means are small and may be wholly a result of chance. If they are real differences, however, they indicate slightly more infections per acre in the intermediate population, with the population extremes close together. Such differences might be attributable to differences in plant susceptibility induced by the different spacings, for spacing did modify growth. Data on fruits and crowns taken during harvest showed that fruit weight diminished as plant population increased. Time of ripening was influenced very little, as was also the apparent quality of the fruit. Crowns, however, were smallest in the closest planting, slightly larger in the most open planting, but still a little larger in the intermediate; and this may have influenced infection inasmuch as crown leaves are susceptible only while elongating and only in the immature zone at the base. There was nothing to suggest that differences in susceptibility were very great.

There had been no important source of vectors within this field. It is presumed, therefore, that thrips were carried in by the prevailing winds from a vacant field located several hundred yards from the nearest corner of this experiment—a field that during and for a time following blossoming of the experimental plants, had supported an abundant growth of weeds.

These data support the hypothesis that infective thrips, blown in from a distance, were scattered almost uniformly over the experimental planting, resulting in approximately equal numbers of infections per unit area but in percentages of infection that are inversely related to plant-population density. Plots with plants 18 inches apart, having only two thirds as many plants per unit area as plots with 12-inch spacing, would be expected to have 50 per cent more infections, a figure reasonably close to the observed value of 57.6 per cent. Although the intermediate spacing gave results not fitting quantitatively so well with expectations, it, too, agrees in showing a lower percentage of infection to accompany dense plant populations, and its departure from expectations is within limits of likely influences of plant susceptibility.—M. B. LINFORD, Pineapple Research Institute, Honolulu, Hawaii.

*Thiosan (Tetramethyl Thiuramdisulfide) and Scurf Control of Sweet Potatoes.*—Since an adequate supply of mercury for fungicidal purposes has become less certain than usual, a new interest in mercury substitutes for

sweet-potato seed and sprout-dip treatments has been aroused. In this connection considerable interest has been shown in Spergon (tetrachloro para benzoquinone). Elmer[1] reports obtaining good control of stem rot by the use of this material. Daines[2] found that Spergon offers only mild control of scurf, while Cook and Harter[3] found that this chemical was not effective for the control of black rot. From these reports it would appear that the use of Spergon on sweet potatoes will be somewhat limited.

During the growing season of 1942, several non-mercurial fungicides were compared with Semesan Bel as sprout dips for the control of scurf (*Monilochaetes infuscans*) and stem rot (*Fusarium* sp.), on the College Farm

TABLE 1.—*Results of sprout treatments for scurf control*

| Treatment | Amount of control | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Underground portion of stem | | | Fleshy roots | | | | |
| | No. examined | Free from scurf | Scurf | Sweet potatoes examined | Free from scurf | Slight scurf | Medium scurf | Unsalable from scurf |
| | | *Per cent* | *Per cent* | *No.* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 1. Semesan Bel, 1 lb. to 10 gal. | 150 | 54.0 | 46.0 | 405 | 49.9 | 22.5 | 7.6 | 20.0 |
| 2. Semesan Bel, 1 lb. to 10 gal.—sprouts held 4 hr. after the dip treatment before planting[a] | 150 | 70.0 | 30.0 | 536 | 66.6 | 14.4 | 6.7 | 12.3 |
| 3. Thiosan, 1 lb. to 7½ gal. | 150 | 56.0 | 44.0 | 408 | 38.2 | 24.8 | 12.0 | 25.0 |
| 4. Thiosan, 1 lb. to 5 gal. | 150 | 61.4 | 38.6 | 457 | 61.9 | 21.0 | 5.7 | 11.4 |
| 5. Spergon (wettable) 12 oz. to 1 gal. | 150 | 34.0 | 66.0 | 404 | 23.2 | 22.3 | 14.9 | 39.6 |
| 6. Untreated check | 150 | 6.0 | 94.0 | 362 | 8.0 | 16.3 | 14.1 | 61.6 |

[a] The sprouts in treatment 2 were wrapped in moist burlap and held for 4 hours before setting in the field. The plants in all other treatments were planted within 15 minutes after the treatment was made.

in New Brunswick. One-half of the Semesan Bel-treated sprouts were planted shortly after they were disinfected, whereas the remaining plants were held for 4 hours following the treatment before being planted. The soil in which the potatoes were grown was a sassafras loam. This soil being heavier than the ordinary sweet-potato soil, favors the development of scurf and renders injury from mercury less likely to occur than would be the case in the sandy loam soils of South Jersey. The sprouts used in this test were grown from nontreated potatoes that were badly affected with scurf.

[1] Elmer, O. H. 1942. Use of Spergon for sweet potato seed and sprout treatment. Pl. Dis. Reptr. 26: 44–46.

[2] Daines, Robert H. 1942. Spergon (Chloranil) and scurf control of sweet potatoes. Pl. Dis. Rptr. 26: 160.

[3] Cook, H. T., and L. L. Harter. 1942. Wettable Spergon not effective as a surface disinfectant of sweet potatoes used for seed. Pl. Dis. Rptr. 26: 222.

Each treatment consisted of a row of 100 plants that was replicated 3 times. Moist soil and warm weather at time of planting favored the rapid establishment of the plants.

An examination of the underground portions of the plants one week after setting, revealed that the sprouts in the Semesan Bel treatments had lost the use of most of the rootlets that were present at setting time. However, new rootlets, as in the other treatments, had already appeared and the aboveground portions of the plants looked as good as those of any treatment. Although injury was present in both treatments where Semesan Bel was used, it was most prevalent in the treatment where the sprouts were held 4 hours after treating before being set in the field. In this treatment approximately 63 per cent of the plants showed browned areas on their stems. Where the plants were set within 15 minutes of the treatment, the browned areas were present on 22 per cent of the stems. The sprouts in all remaining treatments showed no evidence of root or stem injury from the chemical burning, since the stems were free from burned areas and the rootlets that were present at setting time were still functional.

Stem rot was present in only 5 per cent of the nontreated check plants, and although it was less abundant in all the treatments, the incidence of infection was not sufficiently great for the data on stem-rot control to be of much value.

At harvest time data were taken to record the presence or absence of scurf on the underground portions of the stems and on the sweet potatoes themselves. These data are given in the accompanying table.

In this experiment Semesan Bel and Thiosan (tetramethyl thiuramdisulfide) gave good scurf control. This was particularly true for the Semesan Bel-treated sprouts where planting was delayed, and for the 1 to 5 Thiosan treatment. The control provided by Spergon was only moderate, even at the high concentration used.

From the data presented here (Table 1) it would appear that Thiosan 1–5 offers good possibilities as a sweet-potato-sprout dip for scurf control. Under conditions of severe scurf this material was as effective as Semesan Bel. Although the conditions of the test did not favor sprout injury, the data on the injury that did occur justify optimism. One disadvantage with this material in its present state is in its physical properties. Because of its tendency to settle, unusually vigorous agitation of the dipping solution is required.

From the scurf and sprout-injury data obtained from sprouts planted shortly after a Semesan Bel treatment is made as compared with a delayed planting, it would appear that holding sprouts after the treatment increases the fungicidal efficiency of the treatment and also the likelihood of chemical injury.—ROBERT H. DAINES, Agricultural Experiment Station, New Brunswick, N. J.

# REPORT OF THE ANNUAL MEETING OF THE COUNCIL OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY FOR THE YEAR 1942

Results of a questionnaire sent to members in 68 institutions and representing the opinion of approximately 300 members were predominately in favor of cancellation of the 34th annual meeting scheduled for New York, December 27–31, 1942. However, members were greatly in favor of a meeting of the old and new officers and councilmen in conjunction with a meeting of the War Committee of The American Phytopathological Society. Also, members voting were almost unanimously in favor of giving emergency authorization to the Council to make appointments, elect new members, and transact necessary business without immediate approval of the Society—such appointments and business decisions to be immediately effective, but subject to approval of the Society at the next general meeting—all appointments and business decisions to be published in PHYTOPATHOLOGY.

On the basis of this information a business meeting of the Council was held in connection with a meeting of the War Committee, February 12–14, 1943, in the Deshler-Wallick Hotel, Columbus, Ohio.

A conference on "reduced dosages of fungicides and insecticides," previously scheduled for the New York meeting, was held on February 14 with representatives from The American Phytopathological Society, the American Association of Economic Entomologists, and the Biometrics Section of the American Statistical Association participating.

OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1943

**Officers:**

J. C. WALKER, President (1 yr.), University of Wisconsin, Madison, Wisconsin.
J. J. CHRISTENSEN, Vice-President (1 yr.), University Farm, St. Paul, Minnesota.
C. C. ALLISON, Secretary (3 yr. term expires 1944), The Ohio State University, Columbus, Ohio.
H. A. EDSON, Treasurer (3 yr. term expires 1943), Bureau of Plant Industry Station, Beltsville, Maryland.
H. B. HUMPHREY, Editor-in-Chief of PHYTOPATHOLOGY (3 yr. term expires 1945), Bureau of Plant Industry Station, Beltsville, Maryland.

**Councilors:**

J. G. LEACH (term expires 1944), University of West Virginia, Morgantown, West Virginia.
L. M. HUTCHINS (term expires 1943), Bureau of Plant Industry Station, Beltsville, Maryland.
H. A. RODENHISER (term expires 1943), Bureau of Plant Industry Station, Beltsville, Maryland.
O. C. BOYD (New England Division), Massachusetts State College, Amherst, Massachusetts.
G. A. HUBER (Pacific Division), Western Washington Experiment Station, Puyallup, Washington.
A. G. PLAKIDAS (Southern Division), Louisiana State University, University, Louisiana.

**Representatives:**

*A.A.A.S. Council.* L. M. HUTCHINS, J. G. LEACH.
*Division of Biology and Agriculture, National Research Council.* J. C. WALKER (3 yr. term expires June 30, 1946).
*Tropical Research Foundation.* ROBERT D. RANDS (5 yr. term expires 1945).
*Board of Editors, American Journal of Botany.* G. W. KEITT (3 yr. term expires 1943).
*Union of American Biological Societies (and Biological Abstracts).* C. W. BENNETT, DONALD FOLSOM, G. W. KEITT, L. M. MASSEY; H. B. HUMPHREY and C. C. ALLISON (ex officio). F. V. RAND, Chm.

**Standing Committees:**

*Donations and Legacies.* J. G. BROWN, N. J. GIDDINGS, E. C. STAKMAN, N. E. STEVENS, R. P. WHITE, Chm.

*Extension Work and Relations.* S. B. FENNE, R. J. HASKELL, G. W. KEITT, R. H. PORTER, OTTO REINKING, R. C. ROSE, D. R. SANDS, W. B. TISDALE, O. D. BURKE, Chm.

*Investments.* CHARLES BROOKS, MARVIN E. FOWLER, J. W. ROBERTS, H. A. EDSON, Chm.

*Necrology.* M. B. WAITE, A. G. JOHNSON, Chm.

*Phytopathological Classics.* H. H. WHETZEL, Mgr., H. B. HUMPHREY, Editor.

*Public Relations.* L. M. BLANK, O. C. BOYD, K. STARR CHESTER, J. J. CHRISTENSEN, C. T. GREGORY, J. H. JENSEN, FRANK MCWHORTER, A. G. NEWHALL, J. A. PINCKARD, LUTHER SHAW, G. H. STARR, C. E. YARWOOD, G. F. WEBER, Chm.

*Recognition of Merit.* C. R. ORTON, CHARLES CHUPP, J. G. LEACH, L. M. HUTCHINS, H. W. ANDERSON, Chm.

*Regulatory Work and Foreign Plant Diseases.* J. F. ADAMS, A. A. BITANCOURT, A. B. BUCHHOLZ, S. J. P. CHILTON, F. L. DRAYTON, M. R. HARRIS, W. A. MCCUBBIN, Chm.

**Special Committees:**

*Coordination in Cereal and Vegetable Seed Treatment Research.* C. H. ARNDT, H. T. COOK, F. J. GREANEY, C. M. HAENSELER, K. W. KREITLOW, L. D. LEACH, J. H. MCLAUGHLIN, G. L. MCNEW, P. P. PIRONE, H. A. RODENHISER, M. B. MOORE, Chm.

*Fungus Nomenclature.* C. M. TUCKER, D. S. WELCH, ERDMAN WEST, G. L. ZUNDEL, J. A. STEVENSON, Chm.

*International Cooperation and Reorganization.* C. W. BENNETT, J. J. CHRISTENSEN, CHARLES CHUPP, J. A. STEVENSON, J. C. WALKER, FREEMAN WEISS, W. J. ZAUMEYER, L. M. HUTCHINS, Chm.

*Nomenclature and Classification of Plant Viruses.* C. W. BENNETT, E. W. BODINE, EUBANKS CARSNER, F. O. HOLMES, JAMES JOHNSON, FREEMAN WEISS, H. H. MCKINNEY, Chm.

*Publication of Monographs.* H. S. FAWCETT, F. D. FROMME, L. R. HESLER, L. H. LEONIAN, T. F. MANNS, MAX GARDNER, Chm.

*Standardization of Fungicidal Tests.* J. G. HORSFALL, R. W. LEUKEL, J. W. ROBERTS, C. F. TAYLOR, H. W. THURSTON, J. D. WILSON, S. E. A. MCCALLAN, Chm.

*Terminology (Nomenclature) of Immunology and Use of Technical Words.* JESSIE I. WOOD, N. E. STEVENS, Chm.

*War Committee.* E. C. STAKMAN, J. G. LEACH, Acting Chm., R. P. WHITE (Executive Committee).

**Temporarary Committees for 1942:**

*Auditing.* R. D. RANDS, CARL HARTLEY, Chm.

*Resolutions.* N. E. STEVENS, W. G. STOVER, A. E. DIMOND, Chm.

## REPORTS OF OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1942

**Report of the Secretary.** On January 1, 1942, the membership of our Society totaled 1118. At the time of our Council Meeting, February 12, 1943, the membership was 1085. This makes a net loss of 33 members. During the period January 1, 1942, to February 12, 1943, 66 individuals applied for membership. By action of the Council, meeting at Columbus, Ohio, February 12, all applicants were elected to our Society.

Six former members have been reinstated, and the Society lost a total of 104 members: 28 by resignation, 4 by death, and 72 suspended for nonpayment of dues. Of the 72 suspended, 5 were from South America, 1 from Canada, and 29 from other foreign countries. Of the full membership, 155 are paid-up life members, and 1 is paying $10.00 a year toward life membership.

The Secretary here calls attention to the fact that suspended members may be reinstated by notifying the Secretary and paying dues for the current year. Former members should be advised of this and urged to seek reinstatement, if their professional work is directly related to plant diseases and their control.

The Clearing Agency of the Society was continued in 1942. There are 54 applications on hand—16 new ones in 1942, and 6 so far in 1943. Several applications were removed from the Agency because of suspension from the Society for nonpayment of dues. One hundred fifteen applications of 40 pathologists were sent to 21 employers, who requested applications. Three applicants reported they were hired by contact made through the Clearing Agency.

**Report of the Treasurer.** Statement of accounts for the year ending November 30, 1942.

*Receipts:*

| | | | |
|---|---|---|---|
| Balance from 1941 | | | $2569.47 |
| Annual dues: | | | |
| 1941 | $ 34.50 | ($10.00, life) | |
| 1942 | 2592.07 | ( 10.00, life) | |
| 1943 | 1674.75 | ( 10.00, life) | |
| 1944 | 1.00 | | $4302.32 |
| Donations from members for foreign subscriptions | | | 20.00 |
| Balance from A. P. S. dinner in Dallas | | | 21.75 |
| Index payments included in checks for dues | | | 206.00 |
| Sales | | | 2.20 |
| Excess illustrations | | | 9.08 |
| Total receipts | | | 4561.35 |
| | | | $7130.82 |

*Expenditures:*

| | | | |
|---|---|---|---|
| Member subscriptions transferred to PHYTOPATHOLOGY: | | | |
| 1941 | $ 23.50 | | |
| 1942 | 3701.16 | $3724.66 | |
| Transferred to PHYTOPATHOLOGY for: | | | |
| 30-Year Index | 263.00 | | |
| Donations for foreign subscriptions | 20.00 | | |
| Sales | 2.20 | | |
| Excess illustrations | 9.08 | $ 294.28 | |
| Secretarial work and expenses of office of Secretary | | 409.29 | |
| Secretarial work for Treasurer | | 329.19 | |
| Printing | | 189.72 | |
| Stamps and envelopes | | 86.37 | |
| Typewriter and supplies | | 86.73 | |
| Entertainment, dinner in Dallas | | 25.00 | |
| Checks returned by bank | | 19.75 | |
| Miscellaneous: express, telegram, etc. | | 1.55 | |
| Total expenditures | | | 5166.54 |
| Balance on hand | | | 1964.28 |
| | | | $7130.82 |

**Sinking Fund.** The Sinking Fund, the income from which is used for the support of PHYTOPATHOLOGY, has been obtained by deducting $5.00 from each life-sustaining membership installment. All life-sustaining members have paid in full except for one in Italy who, of course, will be unable to complete his payments for the present. The fund totaled $9661.00 at the close of 1941. During the year it increased to $9676.00 and is invested as follows:

| | |
|---|---|
| First mortgage notes deposited with the McLachlen Banking Corporation for collection ($1000.00 at 6%, $500.00 at 5%) | $1500.00 |
| Invested with the following building and loan associations: | |
| Arlington and Fairfax Bldg. and Loan, 4% | 1000.00 |
| Columbia Permanent Bldg. Association, 4% | 500.00 |
| District Bldg. and Loan Association, 4% | 1500.00 |
| National Permanent Bldg. Association, 4½% | 2000.00 |
| Northwestern Fed. Savings and Loan Association, 3½% | 2000.00 |
| Perpetual Bldg. Association, 4% | 1000.00 |
| Prudential Bldg. Association, 3½% (accrued interest, $8.87) | 184.87 |
| | 9684.87 |
| Less interest due PHYTOPATHOLOGY | 8.87 |
| | $9676.00 |

**The Lyman Memorial Fund,** obtained from voluntary contributions, now totals $3132.57. The whole amount is invested with the Brookland Building and Loan Association at 3½%. The account for 1942 follows:

| | |
|---|---|
| Balance on hand, December 15, 1941 | $3181.53 |
| Dividends, Dec. 31, 1941, and June 30, 1942 | 112.02 |
| Contributions from members | 0.25 |
| Sales of Erwin F. Smith Memoir | 5.00 |
| | 3298.80 |
| Accrued dividends transferred to PHYTOPATHOLOGY, Aug., 1942 | 166.23 |
| | $3132.57 |

**Report of the Business Manager of PHYTOPATHOLOGY.** At the close of 1941 there were 584 nonmember subscribers, including 6 complimentary. In 1942 there were 21 cancellations and 186 suspensions for nonpayment, a loss of 207. As there were only 38 new or restored subscriptions, a net loss of 168 resulted. The domestic list remained the same, the number of Pan American subscribers showed a slight increase, but the loss of 78 in the European area and 89 in the Pacific war zone reduced our total list of nonmember subscribers to 416, the lowest since 1925. This loss of subscribers, however, has been partly offset by the purchase by the American Library Association of 45 sets of Volume 32 (1942) of PHYTOPATHOLOGY for future distribution to foreign addresses.

**Statement of accounts for the year ending November 30, 1942.**

*Receipts:*

| | | | |
|---|---|---|---|
| Balance from 1941 | | | $ 5903.33 |
| Subscriptions: | | | |
| 1941 | $ 24.00 | | |
| 1942 | 1993.91 | | |
| 1943 | 323.75 | | |
| 1944 | 5.50 | $2347.16 | |
| Member subscriptions: | | | |
| 1941 | 23.50 | | |
| 1942 | 3701.16 | 3724.66 | |
| Sales of back numbers (including orders for current and back numbers reserved for American Library Association) | | 1032.53 | |
| Advertising: | | | |
| 1941 | 134.85 | | |
| 1942 | 679.06 | 813.91 | |
| 30-Year Index | | 1013.03 | |
| Interest on Sinking Fund | | | |
| First mortgages | 85.00 | | |
| Building and Loan (current and earlier years) | 525.07 | 610.07 | |
| Grant from Rockefeller Institute | | 600.00 | |
| Interest on current funds (current and accrued) | | 415.80 | |
| Interest on Lyman Fund (current and accrued) | | 166.23 | |
| Allowance on reprints | | 591.04 | |
| From authors for excess illustrations | | 158.25 | |
| Payment for article published in July issue | | 92.91 | |
| Payment for reprints | | 26.72 | |
| Drafts and checks redeposited | | 16.50 | |
| Payment for Smith Memoir | | 0.50 | |
| Total receipts | | | 11609.31 |
| | | | $17512.64 |

*Expenditures:*

Printing, distributing and storing PHYTOPATHOLOGY:

| | | | |
|---|---|---|---|
| Vol. XXXI, | No. 11 | $688.58* | |
| | No. 12 and Index | 851.52 | |
| Vol. XXXII, | No. 1 | 864.20 | |
| | No. 2 | 671.44 | |
| | No. 3 | 637.04 | |
| | No. 4 | 776.01 | |
| | No. 5 | 703.59 | |
| | No. 6 | 843.59 | |
| | No. 7 | 799.84 | |
| | No. 8 | 703.75 | |
| | No. 9 | 761.02 | |
| | No. 10 | 681.04 | $8981.62 |

| | | | |
|---|---|---|---|
| Postage, PHYTOPATHOLOGY | 370.84 | $9352.46 | |
| Secretarial work and office expenses, Editor in Chief | | 330.87 | |
| Secretarial work for Business Manager | | 310.00 | |
| Secretarial work for Advertising Manager | | 44.25 | |
| Stamps and stamped envelopes | | 42.96 | |
| Supplies | | 14.69 | |
| Printing | | 38.63 | |
| Postage on 30-Year Index (part) | | 8.18 | |
| Transferred to other accounts: | | | |
| E. F. Smith Memoir | 0.50 | | |
| Science Press, for reprints | 26.72 | 27.22 | |
| Refund, subscriptions | | 25.50 | |
| Checks returned by bank | | 16.50 | |
| Withdrawn from Building and Loan (current funds) for deposit in checking account | | 248.16 | |
| Purchase of early numbers for resale | | 32.50 | |
| Insurance on back volumes | | 10.04 | |
| Telegrams | | 0.59 | |
| Total expenditures | | | $10502.55 |
| Balance on hand: | | | |
| Checking account | | $2510.09 | |
| Northwestern Fed. Savings and Loan Assoc. | | 4500.00 | 7010.09 |
| | | | $17512.64 |

* Total cost, $791.13, less $102.55 paid for article by author.

**The 30-Year Index.** In September, 1941, an edition of 1000 copies was printed. Six hundred and fifty copies have been mailed in response to orders, while 14 copies are being held for future shipment to members and subscribers in occupied or enemy countries. This leaves available a stock of about 335 copies. A summary of receipts and expenses, included in financial reports elsewhere, follows:

| | | |
|---|---|---|
| *Receipts:* | | |
| Sale of index, April, 1941–November, 1942 inclusive | | $3043.78 |
| *Expenses:* | | |
| Honoraria to editor and collaborators | $ 762.50 | |
| Printing and distribution | 2084.50 | |
| Other expenses | 356.55 | $3203.55 |

Accounts payable total $88.00. Against the deficit of $159.77 we have the 350 copies still stored at Science Press.

H. A. EDSON

**Report of the Advertising Manager.** There were 97 revenue-producing advertisements, occupying 61 pages and consisting of 34 full-page, 45 half-page, and 18 quarter-page insertions. During the year 1942, 14 commercial companies used our Journal as advertising medium.

There were 22 nonrevenue-producing advertisements, occupying 12½ pages and made up of notices regarding Phytopathological Classics, the Society Clearing Agency, the 30-Year Index, and a few others.

Contracts in 1942 totaled $973.96.

Kenneth J. Kadow was appointed Advertising Manager at the annual meeting held in Dallas in 1941, and carried on the work through November, 1942, when he resigned to take a position in South America. At the request of the Business Manager, I have since managed the advertising for PHYTOPATHOLOGY.

S. L. HOPPERSTEAD,
*Advertising Manager*

**Report of the Editor in Chief.** Volume 32 of PHYTOPATHOLOGY contains, exclusive of the index, 1100 pages of printed matter and illustrations classified as follows: 120 articles, 46 notes, 4 reports of meetings, 8 book reviews, 137 abstracts, 233 text figures, and 1 frontispiece.

From December 11, 1941, to December 10, 1942, a total of 171 manuscripts of articles, notes, book reviews, and reports of Society meetings were submitted for publication in PHYTOPATHOLOGY. Nine manuscripts were either recalled by their authors or returned to them as unsuitable for publication in our Journal. There are now in press 49 articles and 92 abstracts. Other manuscripts now on hand (Dec. 10) comprise a total of 546 typewritten pages. It is estimated that enough manuscripts have been accepted and are now on file to fill about 720 pages of PHYTOPATHOLOGY.

At this time last year this figure stood at 770, a difference of 50 in favor of 1942. In December 1940 those accepted and awaiting publication were sufficient to fill 820 pages.

Repeated emphasis on the necessity of brevity and conciseness of statement in manuscripts, greater care in the preparation of tabular matter, a more careful choice of and improvement in photographic and other illustrative material, and increased attention to accuracy in preparing citations to literature has borne appreciable fruit. These marked improvements have lessened considerably the amount of editorial work necessary to the preparation of manuscripts for the printer.

The editor takes this opportunity to express his gratitude to members of the Editorial Board and others who from time to time have been called upon for assistance in appraising, constructively criticising, and otherwise improving certain manuscripts that seemed to call for such attention before we could finally decide on what disposition to make of them.

HARRY B. HUMPHREY

**Report of the Manager of Phytopathological Classics for the year 1942.** I beg to submit herewith the annual report of my stewardship as manager of Phytopathological Classics:

Report for the fiscal year beginning December 1, 1941 and ending December 1, 1942

| | | | |
|---|---|---|---|
| Classics No. 1: | On hand 12–1–41 | 65 | |
| | Sold during year | 31 | |
| | On hand 12–1–42 | | 34 |
| Classics No. 2: | On hand 12–1–41 | 267 | |
| | Sold during year | 33 | |
| | On hand 12–1–42 | | 234 |
| Classics No. 3: | On hand 12–1–41 | 362 | |
| | Sold during year | 36 | |
| | On hand 12–1–42 | | 326 |
| Classics No. 4: | On hand 12–1–41 | 425 | |
| | Sold during year | 38 | |
| | On hand 12–1–41 | | 387 |
| Classics No. 5: | On hand 12–1–41 | 672 | |
| | Sold during year | 46 | |
| | On hand 12–1–42 | | 626 |
| Classics No. 6: | On hand 12–1–41 | 793 | |
| | Sold during year | 81 | |
| | On hand 12–1–42 | | 712 |
| Classics No. 7: | On hand 12–1–41 | 0 | |
| | On hand 3–1–42 | 1000 | |
| | Sold during year | 221 | |
| | Gratis copies | 17 | |
| | On hand 12–1–42 | | 762 |

*Receipts:*

| | | |
|---|---|---|
| Cash balance on hand 12–1–41 | $345.14 | |
| Receipts during year | 353.10 | |
| Total | | $698.24 |

*Expenditures:*

| | | |
|---|---|---|
| Postage | $ 14.00 | |
| Express | 1.00 | |
| Bank charges | .85 | |
| General Printing | 21.00 | |
| Classics #7 | 298.17 | |
| Proof reading | 4.50 | |
| Total expenditures | | $339.52 |
| Balance on hand December 1, 1942 | | $358.72 |
| Due on accounts 12–1–42 | | $ 13.00 |

**Report of the Necrology Committee.** In 1942 there were three deaths within our membership as follows:

JOSEPH CHARLES ARTHUR, April 30;
M. MITRA, July 10;
ARTHUR LEWIS PIERSTORFF, July 29.

A. G. JOHNSON
M. B. WAITE

**Report of the American Phytopathological Society Committee on Biological Abstracts and the Union of American Biological Societies.** During America's first year in the war BIOLOGICAL ABSTRACTS has made a magnificent showing in spite of the progressively increasing difficulties stemming from the world catastrophe and involving such vital matters as war censorship; reductions in the number of scientific periodicals, books, and reports from some parts of the world; falling off in subscriptions from the war-torn countries; and excessive turnovers in abstracters, section editors, and supplementary indexers. It is due only to the hard work and able leadership of Drs. Flynn and MacCreight as editors and Mr. Anderson as business manager, together with the full cooperation of a devoted staff and the backing of a sympathetic board of trustees, that so much has been done by so few.

As to subscriptions, a net gain over last year of some 46 to the complete and 86 to the original five sectional editions is reported for the United States. This indicates that Biological Abstracts is filling a real need here and testifies to the firmness of its endorsement by American biologists: this support is, indeed, very good but still not good enough when balanced against the rising costs of publication. Nevertheless, 1942 saw 23,437 abstracts published on 2,377 pages of the ten regular abstract issues of volume 16, a notable achievement in view of the problem of procuring abstracts, particularly of the European literature, under present world conditions. In fact, the continued emphasis and effort towards a fuller abstracting of the biological literature of the world has led to a coverage of about 1700 periodicals in 1942 as compared with 1550 for the preceding year, and greater progress along these lines is hoped for in 1943.

Other advances have included the establishment of a sixth section of Biological Abstracts, Section F—Abstracts of Animal Production and Veterinary Science, which began with January 1942. This new section is not only firmly established but yielded a small profit during the year of its birth. Furthermore, in response to what appeared to be an active demand from many directions, the organization has undertaken during 1942 the promulgation and preparation of a seventh sectional edition, Section G—Abstracts of Food and Nutrition Research, publication of which begins with the issue of January 1943. These two sectional editions are simply rearrangements and reprintings of abstracts also appearing in the complete as well as the original five sectional editions. In this way many biologists who were formerly untouched because there was no one section serving their interests have now been led to subscribe.

During the course of the years Biological Abstracts has come to have the cooperation and collaboration of a very large majority of the active biologists of the United States and many thousands in other countries. The author-abstracting plan is in successful operation for about 250 English language periodicals and has had the supplementary advantage of leading many biologists to volunteer abstracts of their own papers published elsewhere. Special collaborators (listed in the January 1943 issue), each assigned the abstracting of one or more periodicals, now number about 1,100 while over 2,000 others are available for special assignments. There are 150 section editors. Subscriptions total about 3,000. Biological Abstracts has thus become not merely an abstracting journal, but a vast cooperative bibliographic and scientific undertaking, which is steadily developing into an instrument of greater scope and utility.

As to the financial outlook, the picture is less bright but far from black. Although contributions from industrial concerns in 1942 amounted to $3,150 as compared with $700 in 1941, the year ended with a deficit of about $6,000 which must be withdrawn from the very limited reserve funds. There is no assurance that this industrial support will continue. In order to deliver the type of service needed, at least $57,000 in total support for the year should be forthcoming. In view of current trends only about $37,000 can be counted upon from subscriptions. Since this, even with an added $5,000 for the sale of advertising space, still falls short of the lowest estimated needs by $15,000 it is obvious that if standards and coverage are to be maintained, further substantial support from the biological societies, from interested industrial concerns, and from individual biologists generally is an essential desideratum.

Subscriptions to Section D—Plant Sciences (which includes phytopathology and 10 other subject divisions) numbered 335 in the United States during 1942, representing an increase of 23 over the preceding year. Even with the falling off in foreign subscriptions the Section has somewhat better than held its own. This indicates that it is performing a genuine service to plant science and is deserving of still greater support.

For its most healthy advancement science looks well into what has gone before, and now, of all times in history, progress and more progress is the crying need. Here is the only instrument in current existence that serves the whole field of biological research. Biologists: You need Biological Abstracts and Biological Abstracts needs you!

FREDERICK V. RAND, Chairman

Dec. 31, 1942.

**Report of the Society's Representative on the Division of Biology and Agriculture, National Research Council.** It was the major aim of the Division in 1942 to do everything in its power to assist scientists in the fields of biology and agriculture to render maximum service to the war effort. Actions were taken in this direction at the annual meeting in Washington April 11, 1942, and conferences centering around wartime problems were held under Division auspices at various times during the year. This report presents only activities that seem of interest to The American Phytopathological Society.

At the annual meeting a proposal was made for a special committee to head up the wartime activities of the Division. In line with this, E. C. Stakman of this Society was asked to head a conference on Divisional war activities. This conference, attended by 19 biologists, met on August 2. E. C. Stakman and J. G. Horsfall represented the War Emergency Committee of the Society. At this conference the danger of spread by enemy agents of destructive plant and animal pests was brought out. The possibility of serious injury to agriculture also was foreseen from any wartime curtailment of funds for insect-pest and plant-disease surveys or for plant-quarantine enforcement. A real hazard was visualized in the possibility of our armed forces bringing back home dangerous parsites from various parts of the globe, particularly the Pacific area. The conference expressed itself in a motion urging the proper authorities to deal adequately with such wartime problems of protection against foreign insect pests and plant diseases. In view of the need of the Nation for the services of biological and agricultural scientists in the war effort, the establishment of a permanent central committee of the Division was recommended to act as a clearing house for wartime activities.

At the annual meeting of the Division a Crop Protection Committee was established. E. C. Stakman was appointed Chairman. With him J. G. Horsfall and J. G. Leach represent The American Phytopathological Society, and J. L. Horsfall, and W. P. Flint represent the American Association of Economic Entomologists, E. F. Phillips, ex-officio. This committee met on October 12 to consider activities of common interest to both professional groups. In its program the committee decided to work for

(1) the more adequate accumulation of precise information in regard to national and regional plant-disease and insect-pest occurrence and damage as a basis for more effective control programs;

(2) more adequate education of the general public in regard to the menace created by plant diseases and insect pests;

(3) more adequate summarization of important information on crop pests and diseases for the use of extension men and investigators;

(4) the promotion of closer cooperation between entomologists and plant pathologists on a regional and project basis;

(5) closer cooperation between the two profesisonal groups in breeding crop varieties for combined resistance to insect pests and plant diseases;

(6) needed programs for the suppression of important plant diseases and insects;

(7) the wartime continuance of basic research in those fields where interruption would result in disservice to the Nation;

(8) Arrangements for the best possible services with respect to fungicides and insecticides.

At the annual meeting publication was announced of papers read at symposia held by the Committees on Genetics of Pathogenic Organisms and Aerobiology, respectively. Of wide value, these papers include material of phytopathological interest.

The Committee on Maintenance of Pure Genetic Strains indicated that it felt itself under heavy responsibility because of difficulties created by war conditions which might make it hard to preserve all of the needed pure strains of breeding stock.

The discussions at the annual meeting disclosed a large interest in rendering aid to Latin-American Republics in the fields of biology and scientific agriculture.

A National Research Council Fellowship was awarded to B. R. Houston, California Experiment Station, Davis, member of the Society.

Robert F. Griggs, Chairman of the Division, has been actively cooperating with others in working for proper recognition of the functions of professional biological and agricultural scientists in the war effort. In a statement on this subject, issued by the Division, the protection of crops from diseases was listed among the essential contributions to the war effort which require servicing by technical men. Dr. Griggs also has initiated the discussion of ways and means for welding the biological scientists of the country into some sort of strong, coherent, and effective organization.

At the annual meeting H. T. Cook was suggested for membership on the Board of Governors of the Crop Protection Institute. After he was commissioned in the Navy, the Division suggested the name of S. E. A. McCallan in his place.

HOWARD P. BARSS

January, 1943.

**Report of the Tropical Research Foundation.** The Tropical Research Foundation is dissolved and the capital and properties of the Foundation will be disposed of.

R. D. RANDS

**Report of the Committee on Donations and Legacies for the Year, 1942.** This committee has been largely inactive during the year due to the fact that its chairman was unable to attend the Dallas meeting and receive instructions from the Council as to the goal of the committee. The chairman originally and early proposed raising funds for the Endowment Fund through the agency of war bonds and stamps, but funds did not seem to be available for underwriting the effort. Late in the fall a letter was prepared to go to our membership calling attention to the opportunity of supporting both the War Effort and the association through the agency of War Bonds and Stamps. Due to the cancelation of the December meeting in New York this appeal was not sent.

It is suggested to the incoming committee that a real drive be made as proposed in 1943, with the Council appropriating a sum sufficient to underwrite the cost of printing and maintenance, not to exceed $100.

Respectfully submitted,

RICHARD P. WHITE, Chairman

**Report of the Extension Work and Relations Committee.** During 1942 the committee prepared a program for the annual conference sponsored by this group. It was proposed to discuss "What Should Our Major Plant Disease Control Objectives be in 1943?" Due to the postponement of the winter meetings of the Society, the conference was cancelled.

The Extension Subcommittee of the War Emergency Committee of The American Phytopathological Society, selected from the Extension Work and Relations Committee, has used the latter in the furtherance of certain of its projects, i.e., the members were consulted with and contributed to a questionnaire sent to heads of departments of plant pathology throughout the United States. The resulting information will appear in the report of the War Committee.

R. J. HASKELL, G. W. KEITT, R. H. PORTER, OTTO REINKING,, R. C. ROSE,
D. R. SANDS, W. B. TISDALE, S. B. FENNE, O. D. BURKE, Chairman

**Report of the Public Relations Committee for 1942.** During the past year the activities of this committee have been guided by the findings of the Committee of 10, which originally reported (Phytopath. 30: 368. 1940) proposed policies and functions. Each number of the Journal has been carefully worked over by one or more members of the Committee. News-worthy articles have been popularized and disseminated to news writers and certain local publishers with release dates corresponding approximately with the issue of that number.

There has not been evolved a method by which the effectiveness of the work of the Committee can be measured. The personnel of this Committee has been increased and changes are anticipated since it was not selected on a basis of individual interpretation ability. The pathologists who have the writing ability to bridge the gap between the investigator and the layman belong on this Committee; their aid is solicited. Direction, assignments, instruction, organization, complete and prompt responsibility, willing and enthusiastic cooperation, suggestions and constructive criticism dominate the internal activities of the Committee. Liberal and accurate interpretation of original research with full credit has been the principal guide. Certain indications point toward a more inclusive coverage of the United States by including among the disseminating agencies certain newspapers and magazines located in the different parts of the country. The National Association of Science Writers are almost without exception located in the East.

The Committee wishes to recognize the cooperation and sincerely thank the science writers, publishers, officers of the Society and others who have materially aided in accomplishing the work of the Committee during the year.

J. A. PINCKARD, LUTHER SHAW, J. H. JENSEN, C. E. YARWOOD,
J. J. CHRISTENSEN, F. P. MCWHORTER, O. C. BOYD, K. S. CHESTER, P. A. YOUNG,
A. G. NEWHALL, G. H. STARR, C. T. GREGORY, G. F. WEBER, Chairman

**Report of Temporary Committee on Recognition of Merit.** In accordance with the Society's request, the President appointed this committee as a "temporary committee to study the matter of some form of recognition for outstanding papers presented before our Society, and that the papers be considered for the A.A.A.S. prize."

After studying the two phases of this assignment, your committee is impressed with the difficulties of appraising and evaluating contributions, especially on short notice. It is our opinion that any procedure for making awards that requires hurried action would be ill-advised. For example, it does not seem practical, for several reasons, to evaluate abstracts prior to presentation for recognition during the meetings at which they are presented. It would seem much more feasible to give recognition to one or more papers each year by requesting the author to develop his subject further and make a more complete presentation in the form of an invitation paper the following year. This in itself would constitute a recognition of merit and would give time for deliberate judgment as to whether one of these papers might warrant consideration for the A.A.A.S. prize.

To initiate, develop, and carry out some such program it is recommended that a committee be formed that shall consist each year of the last five past presidents of the Society, the chairman being the earliest presidential incumbent in the group, for example, H. W. Anderson would serve as chairman in 1943.

It is further recommended that the framework within which such a committee is asked to function be broad enough to allow for changes in the procedure as the committee gains experience along these lines.

G. H. COONS, H. S. FAWCETT, J. G. LEACH, G. L. PELTIER,
H. S. REED, W. D. VALLEAU, E. B. LAMBERT, Chairman

**Report of the Committee on Regulatory Work and Foreign Plant Diseases.** In accordance with the proposal approved at the Dallas meeting in 1941, a questionnaire was prepared by your committee and submitted to a considerable group of members of the Society especially in touch with various phases of virus disease activities, calling attention to the quarantine aspect of the virus problem as presented by Mr. S. A. Rohwer at Dallas and requesting an expression of opinion on two points.

(1) Which of the courses listed below most nearly represents the attitude you think should be adopted in our national plant quanantine policy toward foreign virus diseases? (The categories are merely suggestive. Your views and comments in any form will be helpful.)
   - A. The exclusion of foreign virus diseases is regarded as impracticable and should not be attempted.
   - B. Efforts should be made to exclude some or a few virus diseases of definitely injurious type and the rest should be disregarded.
   - C. We should aim to protect our important crops against the introduction of foreign virus diseases.
   - D. The proper course is to exclude all foreign virus diseases, to the extent that this can be done without undue disturbance to healthy agricultural development.
   - E. We should endeavor to exclude all foreign virus diseases, by every means in our power, up to the limit of public tolerance.

(2) Keeping in mind the two types of quarantine action mentioned by Mr. S. A. Rohwer, can you propose any specific plant, plant group, or virus complex for which quarantine action involving either host exclusion or entry under observation, or both, would be justifiable?

This questionnaire was sent out April 28, 1942, to 94 members, from whom 19 replies were received. In the first question as to the most desirable national plant quarantine policy toward foreign virus diseases one failed to consider this point and three included two items in the answer. The general summation favors: A, 3; B, 4; C, 5; D, 5; and E, 3. It is thus evident that there was no unanimity of viewpoint on this question and even no obvious trend of opinion which might be helpful to the plant quarantine authorities.

Answers to the second portion of the inquiry were even less complete. Of the 19 replies 3 of the four who touched on it made tentative or exploratory suggestions rather than specific recommendations. Only one of the replies presented anything which could be construed as a recommendation for action, this relating to stone fruits. Others suggested for quarantine consideration were corn, cotton, wheat, truck crops from south of the equator, soy beans, and peanuts. While the net outcome of this inquiry was somewhat disappointing in its tangible results, there was no question of interest and sincerity in the replies, and of the desire of the writers to give the subject helpful consideration.

As an index to the varied trends of thought expressed in these replies, the following summary presents something of the several points of view brought out:

In 8 cases quarantine difficulties were foreseen in regard to symptomless hosts, unknown host range, and multiplicity of hosts, often with different symptoms; the necessity of more energetic study of foreign virus conditions was stressed by 6; two believed a clearing up of our knowledge of the domestic virus situation through plant disease survey activity is a valuable preliminary to attacking the foreign problem; the ineffectiveness of port inspection methods was touched on by 3; the value of domestic control and eradication measures was regarded as high by 3; two called attention to the importance

of excluding foreign insect vectors; two others dealt with the possible factor of virus strains; three saw merit in the proposal to hold certain imported plant materials under detention until their health could be assured; three suggested consideration of foreign certification as a useful if not always satisfactory procedure; in 5 replies comment was made on the national quarantine administration to the effect that present quarantine measures were ineffective for virus diseases, that plant disease activities were not given sufficient consideration, and that its policy should be dynamic rather than static.

Individual comments brought out the following additional viewpoints:

There seems to be no need for a special policy on virus diseases apart from other diseases; the world's virus diseases appear to be already fairly well distributed; the possible importance of a foreign virus disease here is unpredictable; breeding programs for resistance to virus diseases should be extended; delay in introduction of foreign viruses is valuable in giving time for the development of control measures; bringing in foreign viruses for experimental purposes is objectionable; some reliance should be placed on cooperation with foreign countries in the virus problem; it is suspected that strawberry and raspberry diseases have been introduced in plant importations.

A. A. BITANCOURT, A. B. BUCHHOLZ, S. J. P. CHILTON, F. L. DRAYTON, M. R. HARRIS, W. A. MCCUBBIN, Chairman

**Report of the Committee for Coordination of Research in Cereal and Vegetable Seed Treatments.** During the past year this committee has carried on coordinated seed treatment programs with the vegetable crops, cotton, and the small grains including flax. The nature and extent of these programs is reported by the chairmen of the respective subcommittees.

*Vegetable Seed Treatments.* In 1942, the subcommittee on coordination of research on vegetable seed treatments, conducted a series of uniform seed treatment tests with some 30 cooperators in the United States and Canada. Tests were made with peas (George L. McNew), spinach (Harold T. Cook and Richard P. Porter), tomato (L. D. Leach), Lima beans (J. C. Walker and W. W. Hare), Irish potatoes (C. N. Clayton), corn (C. M. Haenseler) and lettuce (C. M. Haenseler). The seed was treated by the leader for each crop (except tomatoes and potatoes) and distributed to interested cooperators, who conducted replicated tests, and reported results to the leader. Complete mimeographed reports of these tests have been or are being prepared by the various leaders and will be distributed to those interested.

There seems to be growing interest in the work on vegetable seed treatments. Thirty-nine cooperators have expressed enthusiasm for the committee's work, and have requested seed samples for trials in 1943. These 39 people have expressed an interest in 19 crops and have requested a total of 207 tests. It is intended that the program should be continued with emphasis upon three war-time objectives: (1) determination of minimum effective dosages for preferred treatments and at least one substitute, nonmetallic compound; (2) location of suitable substitutes among the organic fungicides; and (3) evaluation of secondary substitutes that can be used for emergency adjustments that may be necessary for the 1944 season.

It is anticipated that tests will be made with sweet corn, peas, Lima beans, soy beans, spinach, beets and tomatoes, and Irish potatoes and possibly peanuts.

GEORGE L. MCNEW, Chairman Subcommittee on Vegetables

*Cotton Seed Treatments.* Cooperative plantings to evaluate various treatments of cotton seed were conducted in 8 States in 1942 under the supervision of the Cotton Seedling Disease Committee of the Cotton Disease Council. The treatments included: (a) various kinds of seed; viz., fuzzy, two degrees of reginning, and acid-delinting and (b) sixteen different chemicals and combinations of these chemicals. A mimeographed summary is in preparation and will be distributed to those interested. A special study of the feasibility of reducing the recommended rates of application of Ceresan has been made by R. Weindling in order to determine the possibility of securing the maximal control of seedling diseases with our limited supply of mercury.

C. H. ARNDT, Chairman Subcommittee on Cotton

*Seed Treatments of Small Grains and Flax.* Cooperative trials with wheat, oats, barley, and flax were carried out at 13 stations located in nine Northern States and three Canadian Provinces. Yield tests were made using two seed lots of each of these crops and standard and reduced doses of New Improved Ceresan. Comparisons were made with check plots and in the case of wheat with copper carbonate treated plots. An emergence of seedling-blight control test, using the same seed lots as in the yield tests, was made in which six treatments were compared. In a third series of tests, eight treatments were compared as to their ability to control smuts of wheat, oats, and barley and barley stripe. A mimeographed report of this work is being prepared for distribution.

While reduced facilities at many experiment stations may necessitate the elimination of the yield test it seems desirable to continue the emergence and smut-control tests for extensive testing of new organic compounds and "substitute" fungicides.

M. B. MOORE, Chairman Subcommittee on Cereals

*Other Activities.* Efforts have been made looking toward the organization of seed treatment programs for forage and pasture crops, small grains and sorghum in the Southern States, and corn in the northern and central parts of the Corn Belt. J. Harvey McLaughlin has organized a group for the testing of corn seed treatments in the Southern States and has kindly agreed to associate his group with this committee.

M. B. MOORE, General Chairman

**Report of the Committee on Nomenclature and Classification of Plant Viruses.** In 1941 the Committee expressed approval of the designation of plant viruses by a system of Latin binomials similar to that used for scientific naming of plants. This year discussions have centered around the choice of a system of naming and around the various systems of classification that have been proposed. The Committee favors (Johnson opposed) the use of the Linnean binomial system of nomenclature for plant viruses. A tentative classification based principally on induced symptoms and means of transmission, and consisting of one family and five genera, has been accepted by a majority of the Committee. However, further consultation with other virus specialists is desired before details of the system are completed and prepared for presentation.

Further progress has been made on the compilation of synonyms and descriptions of the viruses affecting the different groups of crop plants. Tentative plans for the publication of these descriptions and lists of synonyms have been formulated.

EUBANKS CARSNER, F. O. HOLMES, JAMES JOHNSON, H. H. MCKINNEY, H. H. THORNBERRY, FREEMAN WEISS, C. W. BENNETT, Chairman

**Report of the Committee on Standardization of Fungicidal Tests.** In continuation of its policy (as outlined in previous reports) of developing standardized methods for testing fungicides in the laboratory and field, your Committee wishes to report that three methods are now in a state suitable for publication. These methods are:

1. Definitions of fungicide terms.
2. The slide-germination method of evaluating protectant fungicides.
3. Standard laboratory Bordeaux mixture.

All methods have been extensively revised since their first appearance as mimeographed "Tentative Methods." The first method or "definitions" in particular has been subject to considerable thought, modification, and revisions. It has been evolved from the constructive criticisms of at least 30 pathologists from 15 different laboratories. The second and third methods include the findings of the recent and extensive research on the subject. It is believed that the three methods will be of assistance as a guide or standard in the testing of fungicides. Accordingly, your Committee recommends that the procedures be published in PHYTOPATHOLOGY under their authorship as "Recommended Methods."

Two tentative methods are being revised for publication as recommended methods. They are:

4. Tentative standardized laboratory and greenhouse procedure for testing the relative fungicidal efficiency of chemical dusts in the control of certain cereal smuts.
5. Tentative recommendations on standard spray nomenclature. This will include Part I—Apples and Peaches, and Part II—Pears and Cherries.

In addition there are in preparation two new tentative methods as follows:

6. Tentative method for testing vegetable seed protectants.
7. Tentative method for testing orchard fungicides.

The Committee would appreciate suggestions regarding new procedures suitable for standardization, as well as criticisms relative to the revision of methods 4 and 5.

Mimeographed copies of the various "Recommended" or "Tentative Methods" may be obtained from the Committee Chairman, Boyce Thompson Institute, Yonkers, New York.

J. G. HORSFALL, K. J. KADOW, R. W. LEUKEL, J. W. ROBERTS, C. F. TAYLOR, J. D. WILSON, S. E. A. MCCALLAN, Chairman

**Annual Report of American Phytopathological Society War Committee.** The formation of the committee was authorized at the Dallas meetings of the Society. There was fairly general unanimity regarding the general scheme of organization, which is based on recognition of the fact that there are regional problems and some of general concern. It seemed wise therefore to organize regional groups, taking advantage insofar as possible of existing divisions of the society, such as the Pacific Coast Division, the Southern Division and the New England Division. A special committee of pathologists

in the Upper Mississippi Valley had already been established under the chairmanship of I. E. Melhus. This group was already functioning well and seemed to furnish a good pattern for other similar groups. Accordingly, regional groups were organized or recognized as follows: New England, Middle Atlantic, Southern, Upper Mississippi Valley, and Pacific Coast. The Canadian Society was recognized, of course, as a coordinate group. The chairman of the divisions or regional chairmen were recognized as representing those groups on the general committee.

All committees have functioned actively. Obviously, however, some of them are of such nature as to permit quicker action than others.

The committees on Quarantines and Plant Disease Survey have been active and have tried to promote action designed to protect the country against the possible introduction of foreign pathogens or exceptionally virulent races of pathogens.

The committee on Extension canvassed the situation with respect to extension facilities in the various states and has attempted to improve the situation where there are not enough extension pathologists.

The committee on Seed Certification has accumulated data regarding seed certification and has sponsored a plan for the requirement of seed treatment for certified seed.

The Fungicide committee has obtained information regarding the availability of fungicides, spray machinery, and other pertinent data with respect to the control of plant diseases by chemical means. In addition, members of the committee have done considerable research on possible substitute fungicides and on the minimum effective dose of well-known fungicides, and have disseminated the information in various ways.

The chairman of each committee made a monthly report, which was sent to all the committee members, including regional representatives, who in turn transmitted this information to so-called "key men" in each State in their region.

Some of the regional groups have functioned extraordinarily well. For example, the Upper Mississippi Valley group has held several meetings; has prepared summaries of plant disease situations; has made further provision for summarized information regarding the control of the most important diseases, with a view to uniformity in recommendations, insofar as possible; and has been generally active in bringing about greater coordination of effort, not only in research, but also in the dissemination of information regarding control measures. The Middle Atlantic group has prepared a list of diseases and control measures for that area which has been mimeographed for general distribution within the area. The Southern group has issued several mimeographed circulars on the control of important diseases.

In general, these regional groups have been very effective in improving the situation with respect to disease control. Although their work has not been spectacular, they have succeeded in bringing about cooperation and coordinated effort within the region. The activities of these groups are fundamental to the effectiveness of the entire organization, and it would be remiss not to commend them for the work that has been done.

On of the most critical situations has been that with respect to manpower. Possibly the situation has been alleviated somewhat through the recognition by the Manpower Commission of the essential nature of the work of "agricultural scientists." Because the importance of plant products now is recognized more than it was at the beginning of the war, it seems likely that the need of well-trained pathologists and the need of replacements will be more clearly recognized in future than it has been in the past; at least there are indications that this is true. The chairman of the committee has exerted considerable effort to bring about alleviation of the situation with respect to personnel in plant pathology. The situation could easily become more critical than it now is, however, and efforts will be continued to bring about official recognition of the need of present plant pathologists and of potential replacements. Because of the provisions of the Selective Service Law, progress along these lines is not so rapid as might be wished; nevertheless, some progress has been made, unfortunately, however, only after too many men already have been lost.

During the past year, relatively little emphasis has been placed on publicity. It seemed desirable to accomplish something first. The time has come, however, when general, regional, and local publicity is highly important. The American people and those in authority owe it to the nation to recognize the importance of maintaining plant public health; and we owe it to the nation to furnish the information.

In addition, it is of the utmost importance to diffuse as widely and as promptly as possible pertinent information regarding plant disease situations and methods of control. Steps have been taken to attain these ends.

The work of the committee during the past year is an indication of what can be accomplished, now that the organization has had experience and has attained some coherence. There is every reason to believe the committee can function much more effectively in the ensuing year than it has during the past year. Although there have been many discouragements, pathologists have a highly essential national service to render; and, despite any resistance or apathy that may be encountered, we must exert every effort of

which we are capable in attempting to attain objectives we know to be in the best interests of the country. And not the least of these is increased effort to promote preventive plant disease measures, not only for the present, but also to safeguard the future.

E. C. STAKMAN, Chairman

**Report of the Auditing Committee, American Phytopathological Society, as of December 1, 1942.** We have examined the books and find them correct, with entries completely supported by vouchers. We wish to congratulate the Business Manager and Mrs. Meier on the clear presentation they have given of the financial transactions.

R. D. RANDS
CARL HARTLEY

**Resolutions of the Committee on Resolutions.** RESOLVED that the Society express to the management and staff of the Deshler-Wallick Hotel and especially to Mr. Tom A. Sahrey and Miss Martha Obetz its sincere appreciation for the courtesy extended to our membership and for cooperation in providing the officers and committees with excellent accommodations for conducting business.

RESOLVED that the Society express its appreciation to C. C. Allison, Secretary, for his continued efficient service to the Society under unusual and trying conditions.

RESOLVED that this Society express its sincere appreciation of the work of the officers and committees in promoting the interests of the Society throughout the year, and especially to the War Committee for its efforts in promoting the agricultural interests of the country.

Respectfully submitted,

NEIL STEVENS
W. G. STOVER
ALBERT E. DIMOND, Chairman

**Elections.** A special committee was appointed by President L. M. Hutchins to canvass the ballots. The ballots were opened and canvassed on December 26 by this committee, the report of which is given on page 84 of the January issue of PHYTOPATHOLOGY.

Sixty-six applications for membership to The American Phytopathological Society were received from January 1, 1942 to February 12, 1943. The Council elected all applicants as members of the Society.

**Reports of Officers, Representatives and Committees.** The reports for the year 1942, as presented on the previous pages, were read and accepted by the Council.

**Appointments and Business Actions.** The Council accepted the resignation of H. B. Humphrey as Editor-in-Chief of PHYTOPATHOLOGY, whose term was to expire December 31, 1943. The Council reappointed H. B. Humphrey as Editor-in-Chief of Phytopathology for a period of three years beginning January 1, 1943. J. M. Hamilton, R. E. Smith, W. W. Wagener, and G. C. Kent were appointed associate editors of PHYTOPATHOLOGY for a term of three years.

S. J. Hopperstead's appointment as advertising manager for PHYTOPATHOLOGY for 1943 was approved, and he was appointed as advertising manager for 1943.

Mrs. Agnes E. Meier was reappointed as assistant business manager of PHYTOPATHOLOGY for 1943.

L. M. Hutchins was appointed as representative to the A.A.A.S. Council for 1943 and 1944.

L. M. Massey for the period 1943 through 1947, and Donald Folsom for the period 1943 through 1948 were appointed as representatives to the Union of American Biological Societies (and Biological Abstracts).

The Council suspended appointment of a representative to the International Union of Biological Sciences.

J. C. Walker was appointed representative of the Society to the Division of Biology and Agriculture, National Research Council for a period of three years beginning July 1, 1943.

J. H. McLaughlin and K. W. Kreitlow were appointed to the special committee on Coordination in Cereal and Vegetable Seed Treatment Research.

J. A. Stevenson was appointed chairman of the special committee on Fungus Nomenclature.

The special committee on Seed Certification was discontinued, since the work of this committee is being taken care of during the emergency by a sub-committee of the War Committee.

The Council approved the appointment of K. Starr Chester, J. J. Christensen, Luther Shaw, and C. E. Yarwood to the standing committee on Public Relations named by

Pres. L. M. Hutchins in 1942. C. T. Gregory and L. M. Blank were appointed to the Public Relations Committee.

The matter of reprinting Classic #1 was left to the judgment of H. H. Whetzel, Manager of Phytopathological Classics.

W. E. Bodine was appointed to the special committee on Nomenclature and Classification of Plant Viruses. H. H. McKinney was appointed chairman of this committee.

The Council approved the report of the temporary committee on Recognition of Merit, and made this a standing committee with the understanding that the membership should rotate and should consist of the five past presidents of the Society, including the most recent ex-president.

H. W. Thurston was appointed to the special committee on Standardization of Fungicidal Tests. The Council recommended that the three procedures developed by the committee on Standardization of Fungicidal Tests be published in PHYTOPATHOLOGY as "recommended methods."

"By action of the Council the policy is established that all articles or abstracts accepted for publication in PHYTOPATHOLOGY, or official reports of committees of the Society, containing 'trade' or 'code' names of pesticides, must divulge in the text or footnotes the chemical composition of said materials."

The Council received a petition for the formation of a Potomac Division of The American Phytopathological Society and voted to submit it without comment to the membership for approval.

The Council acted to change the name of the War Emergency Committee to the War Committee of The American Phytopathological Society.

The Council approved a resolution by F. V. Rand, Chairman of the committee on Union of Biological Sciences and Biological Abstracts urging the Society members in so far as possible to enter and/or keep up their individual subscriptions at least to the Plant Science Section of Biological Abstracts, and to use their influence toward furthering departmental and institutional subscriptions.

The Council approved publication of a membership list of The American Phytopathological Society as a supplement to PHYTOPATHOLOGY as soon as possible.

The decision regarding holding a summer meeting of The American Phytopathological Society was left to the program committee and the executive committee of the War Committee.

The Council recommended that the regular winter meeting be held in conjunction with A.A.A.S., if they meet in Cleveland, Ohio, in 1943, subject to cancellation, if warranted.

The Council advised the Secretary to authorize the use of the membership list to the Agricultural Insecticide and Fungicide Association with the stipulation that the list would be used only for sending AIF News, and would NOT be available to firms comprising the Agricultural Insecticide and Fungicide Association.

# JOSEPH CHARLES ARTHUR

January 11, 1850–April 30, 1942

Joseph Charles Arthur was graduated from the Iowa State College in 1872 with the degree of Bachelor of Science, and received the degree of Master of Science from the same institution in 1877. He was granted the degree of Doctor of Science by Cornell University in 1886. His honorary degrees included the Doctor of Laws, University of Iowa, 1916; Doctor of Science, Iowa State College, 1920, and Purdue University, 1931. He also pursued graduate study at Johns Hopkins and Harvard in 1879 and at Bonn in 1896.

In 1884, he was appointed botanist at the Agricultural Experiment Station, Geneva, New York. This was the first appointment to such a position in this country. In 1887 he was called to Purdue University as Professor of Botany. The following year his title was changed to Professor of Vegetable Physiology and Pathology and Botanist to the Indiana Agricultural Experiment Station. This position he held until his formal retirement in 1915. From then until his death he was Emeritus Professor of Botany. In the years between his graduation and his going to Geneva it was his determination to make botany his life work, but botanical positions were few. For a while he had no work: he taught country schools during several winters; he returned to the college at Ames several times to carry on special work; and was instructor at the Universities of Wisconsin and Minnesota during part of the time.

Dr. Arthur was a charter member of the American Phytopathological Society, and was a member of numerous other American and foreign scientific societies.

Although an important and pioneer contributor to several botanical fields, particularly physiology and pathology, his great life work was with the Uredinales. He was an inspiring leader in the investigation of the rusts for more than fifty years. From the time of his first rust papers in 1883 to his last papers in 1936, he was studying the life-cycles, relationships, classification and distribution of these fungi. The results are recorded in 150 papers and three major publications. He was much interested in the rules of nomenclature and made numerous trips to Europe to confer with fellow workers and to attend International Congresses. Throughout an unusually long life Dr. Arthur was productive not only through his own unceasing efforts but also through influence on the lives of students, assistants, correspondents and associates.

## MANORANJAN MITRA

June 19, 1895–July 10, 1942

Manoranjan Mitra was graduated from Punjab University (Government College), Lahore, India, in 1916 with the degree of Bachelor of Science, and in 1918 he received the degree Master of Science from the same institution. He was granted the Diploma of the Imperial College of Science (London) in 1929 and the degrees of Doctor of Philosophy (1929) and Doctor of Science (1936) from London University.

For a time, Dr. Mitra was a Lecturer in St. John's College (Agra, India), but as his interests lay in research he went to the Imperial Agricultural Research Institute at New Delhi, India, first in 1919 as a graduate student and later as an associate. Soon after completing his training at London University, Dr. Mitra was appointed Assistant Mycologist at the Imperial Agricultural Research Institute (New Delhi, India), which position he held until the time of his death.

Dr. Mitra was a man of retiring yet friendly nature. He was especially studious and gained an extensive knowledge of plant diseases in India. He was a scholarly teacher and a most congenial associate and loyal friend.

## ARTHUR LEWIS PIERSTORFF

November 12, 1895–July 28, 1942

Arthur Lewis Pierstorff was graduated from The Ohio State University in 1919 with a degree of Bachelor of Arts, and received the degree of Doctor of Philosophy from Cornell University in 1929.

Dr. Pierstorff was assistant county agent at Rochester and Fredonia, New York, 1921–1922; research fellow at Cornell University 1922–1923; plant disease director of the New York State syray service at Cornell University 1924–1926; extension horticulturist at Rutgers University, New Jersey State College of Agriculture 1926–1927; extension plant pathologist and professor of botany at The Ohio State University, 1928–1938; and professor of botany in the same institution from 1938 to the time of his death. He was a second lieutenant, field artillery, U. S. Army in 1918.

Dr. Pierstorff was a recognized authority in the field of plant pathology and pomology. He organized a centralized scab spray service for the State of Ohio, which has been an outstanding service to the orchardists of the State. The effects of his very capable and efficient service in extension work will long continue to be evident in all parts of the State. His affable, sincere, and genuine manner, and his warm personality won him many friends among his colleagues and among the agriculturalists of the State.

# VAPOR ACTION OF CERTAIN FUNGICIDAL MATERIALS PREPARED FOR DUSTING COTTON SEED[1]

S. G. LEHMAN

(Accepted for publication September 24, 1942)

In recent years certain commercial preparations having fungicidal properties have been widely recommended for dusting seeds before planting. Numerous workers have shown great reduction in smut of wheat (9), oats (10, 15), and barley (8) and marked improvement in cotton seedling emergence and survival (1, 4, 7, 11, 12, 14) as a result of treating seed with copper, zinc, and mercury dusts. The first dust preparation to be widely used on cotton seed was Ceresan. Ceresan, as prepared at present, contains 2 per cent of ethyl mercury chloride as its active ingredient. A second preparation, introduced for use in control of seed-borne parasites of cereals and since found to be very effective in control of fungi found on cotton seed, is sold under the name New Improved Ceresan. This preparation contains 5 per cent of ethyl mercury phosphate as its active ingredient. Another preparation of considerable merit for dust treatment of cotton seed is known to the trade as Sanoseed. This preparation is said by the manufacturer to contain not less than 2 per cent of ethanol mercury chloride.

Recommendations for seed treatment usually have emphasized the importance of using enough of the chemical and of thorough mixing of chemical and seed so that each seed becomes coated with particles of the disinfectant. With non-volatile disinfectants this would seem necessary. However, consideration of availability and cost of materials and of the toxic effect of the chemicals on the seed itself makes the use of small quantities desirable. A gaseous fumigant might have considerable advantage over one of low volatility in that the former, although used in small doses, could reach spores lodged in minute crevices impenetrable by fungicides in water solution.

When New Improved Ceresan is used for cotton, 1 and ½ oz. usually is recommended as the quantity to use for 1 bushel of fuzzy seed. In the writer's experiments in which this dust was applied in different quantities to cotton seed infested with the anthracnose fungus, *Glomerella gossypii,* and the pink boll rot fungus, *Fusarium moniliforme,* application of as little as ½ or ¼ ounce per bushel of seed usually has given nearly as great improvement in seedling emergence and survival as the use of larger quantities. It seems improbable that so little as ½ oz. of this dust containing only 0.025 oz. of active ingredient could by the methods employed be so uniformly distributed over 30 lb. of fuzzy seed as to bring a fungicidally effective dose in direct particle contact with more than a relatively small proportion of the spores on the seed. It was suggested that the effectiveness of this dust in

[1] Contribution from the Department of Botany, North Carolina Agricultural Experiment Station. Published with the approval of the Director as Paper No. 142 of the Journal Series.

small dosages is due to the volatility of the mercurial ingredient. It has been shown that certain microorganisms (5) and seed plants (6,-16) and human beings (3) may be harmfully affected by vapors arising from mercury or its compounds and that formaldehyde vapor (2) is an effective agent for control of fungi on seeds. With a seed like cotton, which normally has its surface covered with short lint, a volatile fungicide should have a marked advantage over one of no volatility. This paper gives an account of experiments made to test the vapor action of mercurial preparations used for treating cotton seed.

## EXPERIMENTS WITH SEEDS

In these experiments both naturally infested and artificially inoculated seeds were used. All broken and visibly defective seeds were removed in order to reduce as much as possible the inaccuracies that might arise from inclusion of defective seeds. The sound seeds reserved for use were thoroughly mixed and then separated into equivalent lots for treatment. In making the treatments, a weighed quantity of the chemical preparation to be used was spread in a thin layer over the bottom of a large glass dish. Sufficient time was allowed for all dust to settle. The seeds were laid on wire racks above the chemicals. In some experiments the treatments were made in desiccator dishes with vapor-tight lids; in others, large 3-liter capacity culture dishes with well fitted but unsealed lids were used. Control lots were treated similarly, except for omission of the fungicide from the dishes. The treated seeds were germinated either in steamed sand in flats in the greenhouse or on paper toweling in large glass moist-chamber dishes in the laboratory. Fifty seeds were taken as the planting unit for each sublot, and each planting was replicated 4 or 8 times. The sublots of each test were randomized in the plantings. Other details of the treatments will be given as the various experiments are described.

In experiment 1 (Table 1), seeds naturally infested with *Glomerella gossypii* and *Fusarium moniliforme* were exposed to vapor from New Improved Ceresan. In experiment 2 (Table 1), both Ceresan and New Improved Ceresan were tested. Such an amount of each dust preparation was used as would give equivalent weights of the active ingredients. The seeds were germinated in steamed sand. In both experiments, the control lots gave a low percentage of disease-free plants, while the lot stored above Ceresan and New Improved Ceresan and the lot in which Ceresan was applied directly to the seed gave high percentages of disease-free seedlings. Obviously both Ceresan and New Improved Ceresan contain substances whose vapors are fungicidally active toward *G. gossypii* and *F. moniliforme* present on seed.

The treatments described above were made at laboratory temperatures which varied from 20° C. upward. It was of some interest to know whether similar effects would follow treatment at a lower temperature. Table 2 gives results of a test (Exp. 1) in which naturally infested seed was treated at a uniform temperature of 14° C. and at 28° C. The seeds were germinated

on paper toweling in large moist chamber dishes. This method of germination made it possible to identify the fungi present by direct examination of the diesased seedlings. *Fusarium moniliforme* and *Glomerella gossypii* were present on nearly all of the diseased seedlings. Seeds treated with vapors from New Improved Ceresan at 14° for 24 hours showed a higher percentage of germination and a much lower percentage of diseased seedlings than the untreated control. The 48- and 72-hour exposure periods gave disease control that was somewhat better than the 24-hour period, but not so good as that obtained by direct application of the dust to the seed. Exposure at 28° C. appears to have given somewhat better control of seedling diseases than at 14° C. Other similar tests not being presented here show good vapor action of New Improved Ceresan at 18° C. for exposure periods of 24 and 48 hours.

TABLE 1.—*Number and percentage of diseased seedlings arising from cotton seeds naturally infected with Glomerella gossypii and Fusarium moniliforme and subjected to vapors arising from Ceresan and New Improved Ceresan*

| Experiment No. | Lot | Treatment | Seedlings free of disease | |
|---|---|---|---|---|
| | | | Number[a] | Percentage seeds planted |
| 1 | A | Control; stored 3 days in empty dish | 1.6 | 3.2 |
| | B | Stored 3 days over New Improved Ceresan[b] | 47.4 | 94.8 |
| | C | Control; stored 16 days in empty dish | 2.6 | 4.2 |
| | D | Stored 16 days over New Improved Ceresan[b] | 47.5 | 95.0 |
| | | Difference required for significance, odds 99 to 1 | 3.6 | 7.2 |
| 2 | A | Control; stored 2 days in dish without Ceresan | 8.3 | 16.6 |
| | B | Stored 2 days over 15 gms. of Ceresan[c] | 34.4 | 72.8 |
| | C | Stored 2 days over 6 gms. of New Improved Ceresan[b] | 46.5 | 93.0 |
| | D | Seed dusted with Ceresan[c] | 46.1 | 92.2 |
| | | Difference required for significance, odds 99 to 1 | 2.6 | 5.2 |

[a] Mean of 8 replications of 50 seeds each.
[b] 5 per cent ethyl mercury phosphate.
[c] 2 per cent ethyl mercury chloride.

In other experiments vapor arising from Sanoseed was tested against *Glomerella gossypii*. Old seeds known to be free of natural infestation of *Fusarium moniliforme* were artificially inoculated with conidia of *G. gossypii*. The air-dry inoculated seeds were exposed to vapor from 5 g. of Sanoseed in large culture dishes, held at 3 constant temperatures. The results are given as Exp. 2 and 3 in table 2. Experiment 2 was germinated on paper toweling in moist chamber dishes at room temperature. A significantly smaller proportion of the seedlings became diseased from seeds, exposed to Sanoseed at 28° than at 5° C. However, this decrease appears to have been due to an adverse effect of drying the conidia on the seeds held at 28° C. The percentage of diseased seedlings from seeds exposed to Sanoseed at 38° is less by low odds than from seeds of the comparable untreated control.

TABLE 2.—*Effect of subjecting cotton seed naturally and artificially infested with G. gossypii to vapor from seed treatment preparations at different temperatures and for different periods of time*

| Experiment No. | Treatment: Preparation used | Temperature ° C. | Time hrs. | Germination as percentage seeds planted[a] | Diseased seedlings[b]: as percentage seeds planted | Diseased seedlings[b]: as percentage seeds germinated |
|---|---|---|---|---|---|---|
| 1[d] [g] | Control, no fungicide used | Room | | 59.5 | 43.5 | 73.1 |
| | New Imp. Ceresan,[j] vapor | 14 | 24 | 74.0 | 12.5 | 16.9 |
| | " " " " | " | 48 | 88.5 | 4.5 | 5.1 |
| | " " " " | " | 72 | 82.5 | 4.0 | 4.8 |
| | " " " " | 28 | 48 | 82.0 | 2.0 | 2.4 |
| | " " " dust on seeds | Room | [c] | 79.5 | 0.0 | 0.0 |
| | Diff. req. for signif.; odds 99 : 1 | | | 14.1 | 5.7 | |
| 2[d] [h] | Control, no fungicide used | 28 | 72 | 87.0 | 70.5 | 81.0 |
| | Sanoseed,[i] vapor | 5 | " | 88.0 | 81.5 | 92.6 |
| | " " | 28 | " | 84.5 | 69.0 | 81.7 |
| | " " | 38 | " | 74.5 | 56.0 | 75.2 |
| | Control, no fungicide used | 38 | " | 74.5 | 62.5 | 83.9 |
| | Diff. req. for signif.; odds 99 : 1 | | | 10.2 | | 10.2 |
| 3[e] [h] | Control, no fungicide used | 18 | 72 | 6.5[f] | 6.5 | 100.0 |
| | Sanoseed, vapor | 18 | " | 17.5 | 16.5 | 94.3 |
| | Control, no fungicide used | 28 | " | 11.0 | 11.0 | 100.0 |
| | Sanoseed, vapor | 28 | " | 6.5 | 6.5 | 100.0 |
| | Control, no fungicide used | 38 | " | 11.0 | 10.5 | 95.5 |
| | Sanoseed, vapor | 38 | " | 15.5 | 15.5 | 100.0 |
| | Ceresan,[k] vapor | 18 | " | 83.5 | 0.0 | 0.0 |
| | " " | 28 | " | 88.0 | 0.0 | 0.0 |

[a] Percentage is based on 200 seeds, the number planted for each treatment.
[b] *Glomerella gossypii* and *Fusarium moniliforme* in experiment 1, *G. gossypii* alone in experiments 2 and 3.
[c] Dust applied to seeds about 1 hour before planting.
[d] Seeds germinated on paper in culture dishes.
[e] Seeds germinated in steamed sand in greenhouse.
[f] Figures represent per cent seeds emerged, total germination not determined on experiment 3.
[g] Seeds naturally infested with *G. gossypii* and *F. moniliforme*.
[h] Seeds free of *F. moniliforme* but artificially inoculated with *G. gossypii*.
[i] Contains 2 per cent of ethanol mercury chloride.
[j] Contains 5 per cent of ethyl mercury phosphate.
[k] Contains 2 per cent of ethyl mercury chloride.

In experiment 3 (Table 2) the seeds were planted in steamed river sand in the greenhouse. Total germination was not determined but emergence and disease records were taken. No vapor action of Sanoseed against *Glomerella gossypii* was indicated. Most of the seedlings died before emergence. The small proportion that did emerge died soon afterward or showed pronounced anthracnose lesions on the stems. Vapor from Ceresan, however, gave high seedling emergence and complete freedom from disease. These results (Exp. 2 and 3), which are supported by a preliminary experiment not given here, indicate that vapor action by Sanoseed is only feebly if at all effective at ordinary temperatures.

Old seeds or seeds that have been improperly stored often are found infested with and susceptible to injury by such molds as *Rhizopus nigricans, Cephalothecium roseum,* and *Aspergillus* sp., fungi not usually regarded as important parasites of cotton seedlings. Seed of this character, even though free of the more virulent parasites, *Glomerella gossypii* and *Fusarium moniliforme,* is often greatly improved in respect to seedling emergence by dusting with a mercury fungicide before planting in the field. A preliminary experiment with old seeds exposed to vapor of New Improved Ceresan indicated considerable reduction in seedling mortality from such treatment. To establish this point additional experiments were made using seed approximately 5 years old and known to be infested by and susceptible to *R. nigricans.* A brown-spore and black-spore species of *Aspergillus* were also on the seeds. In the course of these experiments, the temperature, period, and manner of exposure were varied somewhat, and several dust preparations were used. The seed was germinated on paper toweling in large moist-chamber dishes in order better to observe the fungi developing on the seed and seedlings. Two hundred seeds were germinated for each treatment. After germination began, records were taken at daily intervals, and diseased seedlings were removed as soon as the causal fungi could be recognized. Certain details and the results of these treatments are given in table 3.

In experiment 1 (Table 3), molds, chiefly Rhizopus, destroyed 43.5 per cent of the seeds of the control, either before or soon after germination. Storage in vapor above ethyl mercury phosphate greatly reduced the percentage of moldy and damaged seedlings and increased the percentage of uninjured seedlings. Treatment at 28° C. gave markedly better results than at 5° C. Application of the dust directly to the seeds entirely inhibited growth of the seed-molding fungi.

In experiments 2 and 3 (Table 3), several seed-treatment preparations were used and such an amount of each preparation was stored in the dishes with the seeds as to give an equal weight of the active ingredient for each preparation tested. Thus, if the concentrations of active ingredient as given by the manufacturer were correct, the seeds in each treated lot were exposed to vapors from 0.1 g. of the chemical ingredient. Best results from the vapor treatments were obtained by use of the preparation containing ethyl mercury chloride. Not only were fewer seeds destroyed by mold fungi

TABLE 3.—*Effect of subjecting cotton seed infested with Rhizopus, Aspergillus and other mold fungi to vapors arising from seed-treatment preparations containing mercury in organic combination*

| Experiment No. | Treatment | | | | | Seeds moldy; not germinating, or radicles diseased soon after germination[a] | Seeds moldy; germinating, radicles not injured[b] | Seeds germinating, radicles not injured[c] |
|---|---|---|---|---|---|---|---|---|
| | Active ingredient and percentage in preparation used | Amount of preparation used | Time | Temperature | Receptacle | | | |
| | | *Grams* | *Hours* | *° C.* | | *Per cent* | *Per cent* | *Per cent* |
| 1 | Control; no chemical | ..... | 72 | Room | Paper bag | 43.5 | 18.0 | 52.0 |
| | Ethyl mercury phosphate, 5 per cent | 5 | 72 | 5° C.[e] | Covered dish | 27.5 | 26.5 | 73.5 |
| | Ethyl mercury phosphate, 5 per cent | 5 | 72 | 28° C.[e] | " " | 1.5 | 0.0 | 91.0 |
| | E-M-P dust on the seed, 5 per cent | [d] | 72 | Room | Paper bag | 0.0 | 0.0 | 91.5 |
| | Diff. req. for signif., odds 99 : 1 | ..... | ..... | ..... | ..... | 10.7 | ..... | ..... |
| 2 | Control; no chemical | ..... | 50 | 22[f] | Paper bag | 47.5 | 4.5 | 50.5 |
| | Ethyl mercury chloride, 2 per cent[g] | 5 | 50 | " | Covered dish | 3.5 | 0.5 | 94.5 |
| | Ethyl mercury phosphate, 5 per cent[h] | 2 | 50 | " | " " | 24.5 | 16.0 | 75.0 |
| | Ethyl mercury borate, 5 per cent[i] | 2 | 50 | " | " " | 19.5 | 13.5 | 80.0 |
| | Ethyl mercury iodide,[i] 5 per cent | 2 | 50 | " | " " | 7.5 | 35.0 | 91.5 |
| | Ethanol mercury chloride, 2 per cent | 5 | 50 | " | " " | 52.0 | 2.0 | 48.0 |
| | Ethanol-M-C dust on seed, 2 per cent[j] | [d] | 18 | " | Paper bag | 3.5 | 0.0 | 96.0 |
| | Diff. req. for signif., odds 99 : 1 | ..... | ..... | ..... | ..... | 16.8 | ..... | ..... |
| 3 | Control; no chemical | ..... | 73 | 26[f] | Paper bag | 38.0 | 10.0 | 47.0 |
| | Ethyl mercury chloride, 2 per cent | 5 | 73 | " | Covered dish | 2.0 | 0.0 | 87.5 |
| | Ethyl mercury chloride, 2 per cent | 5 | 73 | " | Sealed dish | 2.0 | 1.0 | 86.5 |
| | Ethyl mercury phosphate, 5 per cent | 2 | 73 | " | Covered dish | 17.5 | 27.5 | 73.5 |
| | Ethyl mercury phosphate, 5 per cent | 2 | 73 | " | Sealed dish | 16.5 | 40.0 | 69.5 |
| | Ethyl mercury borate, 5 per cent | 2 | 73 | " | Covered dish | 20.5 | 27.5 | 69.5 |
| | Ethyl mercury iodide, 5 per cent | 2 | 73 | " | " " | 3.5 | 43.0 | 86.5 |
| | Ethanol mercury chloride, 2 per cent | 5 | 73 | " | " " | 43.5 | 9.5 | 49.0 |
| | E-M-borate dust on seed, 5 per cent | [d] | 73 | " | Paper bag | 0.0 | 0.0 | 87.5 |
| | Diff. req. for signif., odds 99 : 1 | ..... | ..... | ..... | ..... | 14.8 | ..... | ..... |

[a] *Rhizopus nigricans* on most of the seeds; a brown-spore *Aspergillus* second in importance, *Cephalothecium roseum* and *Penicillium* present but of minor importance.

[b] A black-spore *Aspergillus*, apparently entirely non-parasitic, was the chief fungus present on the seed coats.

[c] Includes percentages given in column (b) but none in column (a).

[d] All that will adhere to seed after shaking lightly on a screen.

[e] In constant temperature chambers.

[f] In warm room, the mean temperature for the period of exposure.

[g] Ceresan.

[h] New Improved Ceresan.

[i] Experimental preparation, no established trade name.

[j] Sanoseed.

(*Rhizopus nigricans* and the brown-spore *Aspergillus*), but also the vapor almost completely prevented growth of the non-parasitic black-spore *Aspergillus* on the coats of germinating seedlings. Ethyl mercury iodide was second in respect to effectiveness of its vapors in preventing *Rhizopus* and the brown-spore *Aspergillus* from destroying seeds and seedlings. However, in control of the black-spore *Aspergillus* on the coats of germinating seeds, the ethyl mercury iodide preparation was least effective of any tested. Some selective action between fungi and chemical seems to be indicated. Ethyl mercury phosphate and ethyl mercury borate preparations were each about equally effective in respect to the fungicidal action of their vapor. Each also was significantly less effective than ethyl mercury chloride against all fungi on the seeds. The least effect on seed-destroying fungi was obtained from vapor from ethanol mercury chloride. This material is obviously of low volatility. The same material applied directly to the seeds, however, gave good control of fungi on the seeds.

In experiment 3 (Table 3), comparisons were made between treatments in dishes having ground glass lids sealed with petrolatum and in culture dishes having well fitted but unsealed lids. No advantage was found in the use of a sealed dish as compared to a covered, unsealed dish. Apparently the quantities of material used (0.1 g. of active ingredient) was great enough to maintain an atmosphere approximately saturated with chemical vapor in the covered, unsealed dishes.

### EXPERIMENTS WITH CONIDIA

In addition to experiments with infested cotton seed other experiments were carried out in which conidia of *Glomerella gossypii* were exposed to vapors from various chemical preparations. A culture of the fungus isolated from a diseased cotton seedling and known to be pathogenic was grown on potato-dextrose agar in Petri dishes until an abundance of spores had formed. The surface of the dishes was rinsed with water to get a heavy suspension of conidia. Two 3-mm. loopfuls of this conidial suspension were placed on small squares of sterile filter paper in sterile Petri dishes. The Petri dishes containing the inoculated filter paper squares were placed in a cupboard with the lids removed until the paper and spores had become air-dry. The dry inoculated papers were exposed to the vapors of the chemicals in the manner described above for exposure of seeds; *i.e.*, by placing an open Petri dish in a closed culture dish over the chemical being tested. In some instances instead of exposing inoculated papers, the conidia were placed on the papers after exposure to the chemicals. Glass cover slips were substituted for the paper in some experiments. After the treatment these were placed on water agar containing 0.1 per cent dextrose to determine if the treated conidia were still viable. For convenience the paper squares and glass cover slips with conidia will be referred to as "cultures."

Preliminary tests indicated that conidia of *Glomerella gossypii* on paper squares could be killed by exposure to vapor from 2 g. of New Improved

TABLE 4.—*Effect of exposing air-dry conidia of Glomerella gossypii on filter paper to vapor emanating from preparations containing mercury in organic form*

| Exp. No. | Treatment: Active ingredient and percentage in preparation used | Amount of preparation used g. | Length of gassing period (hours): 0 | 1 | 2 | 3 | 4 | 6 | 12 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Number of cultures alive out of 5 gassed | | | | | | | |
| 1 | Control | 0 | 5 | | | | | | | |
| | Ethyl mercury phosphate, 5 per cent | 2.0 | | | | | | 0 | 0 | 0 |
| | Ethyl mercury phosphate, 5 per cent | 1.0 | | | | | | | | 0 |
| | Ethyl mercury phosphate, 5 per cent | 0.5 | | | | | | | | 0 |
| | Ethyl mercury phosphate, 5 per cent | 0.25 | | | | | | | | 0 |
| 2 | Control | 0 | 5 | | | | | | | |
| | Ethyl mercury phosphate, 5 per cent | 2.0 | | 1 | 0 | | 0 | 0 | | |
| 3 | Control | 0 | 5[a] | | | | | | | |
| | Ethyl mercury chloride, 2 per cent | 5 | | 0 | 0 | 0 | 0 | | | |
| | Ethyl mercury phosphate, 5 per cent | 2 | | 0 | 0 | 0 | 0 | | | |
| | Ethyl mercury borate, 5 per cent | 2 | | 5 | 2 | 1 | 0 | | | |
| | Ethyl mercury iodide, 5 per cent | 2 | | 1 | 0 | 0 | 0 | | | |
| 4 | Control | 0 | 5[b] | | | | | | | |
| | Ethyl mercury chloride, 2 per cent | 5 | | 0 | 0 | 0 | 0 | | | |
| | Ethyl mercury phosphate, 5 per cent | 2 | | 2 | 0 | 0 | 0 | | | |
| | Ethyl mercury borate, 5 per cent | 2 | | 5 | 2 | 0 | | | | |
| | Ethyl mercury iodide, 5 per cent | 2 | | 0 | 0 | 0 | 0 | | | |

[a] Mean of 4 control plates of 5 cultures each.
[b] Mean of 2 control plates of 5 cultures each.

Ceresan for a period of 24 hours. Table 4 gives results from other experiments using different seed treatment preparations, different weights of certain of the preparations, and different exposure periods. In experiments 1 and 2 as little as 0.25 g. of the ethyl mercury phosphate preparation provided sufficient vapors to prevent growth of cultures after 24 hours of gassing. Exposure as short as 2 hours to 2 g. resulted in failure of all cultures to grow.

Comparative tests of vapor action of 4 seed-treatment preparations were made in experiments 3 and 4 (Table 4). Such a quantity of each preparation was taken as would put an equivalent amount (0.1 g.) of the active ingredient in the gassing chambers (large, unsealed culture dishes). Treatments were continued from periods of 1, 2, 3, and 4 hours at a temperature of 28° C. No cultures grew after 1 hour's exposure to the preparation containing ethyl mercury chloride. The preparations containing ethyl mercury phosphate and ethyl mercury iodide were apparently somewhat less effective than ethyl mercury chloride. Of the 4 preparations applied ethyl mercury borate was least effective as a volatile agent, more than 2 hours' exposure being required to inhibit growth of all cultures by this material.

Other experiments were made in which air-dry conidia were gassed at different temperatures by vapors from ethyl mercury chloride, ethyl mercury phosphate, and ethanol mercury chloride (Table 5). At all temperatures used (5, 18, and 28° C.) vapors from the ethyl mercury chloride completely inhibited growth of the filter-paper cultures. The ethyl mercury phosphate preparation was somewhat less effective, only partly inhibiting growth for the 2- and 4-hour treatment periods at 5° C. Growth was prevented completely when treatments were made at 28° C. Ethanol mercury chloride was much less effective in vapor action than the other materials used. Growth occurred from all cultures except one exposed to this material.

In the experiments described above, growth of the conidia exposed to vapors of certain organic mercury compounds was inhibited. Assuming that the chemical vapors condense on the conidia and paper and become fungicidally active only when the cultures are put on moist media for germination, one might expect those vapors that had condensed on the conidial cultures from a saturated atmosphere in a given period of exposure would in a like period harmlessly volatilize from the cultures when they were moved to open air. Table 6 gives results of exposing air-dry conidia on filter paper to vapors of 4 organic mercury preparations for 1, 3, 4, and 24 hours and subsequently moving them to open air to permit escape of the material that may have condensed on them. No growth occurred after 24 hours' exposure to any of the 4 chemicals, even though such exposure was followed by a 24-hour period of airing to permit volatilization and dissipation of condensed vapors adhering to the surface of paper and conidia. With ethyl mercury chloride and ethyl mercury phosphate, growth

of all cultures was inhibited by 3 hours of gassing; but 24 hours of subsequent airing failed to remove the inhibiting agent. A period of airing considerably longer than the period of exposure was required to remove the inhibiting chemical from the cultures; and increasing the period of exposure of the cultures to the vapors increased the difficulty of freeing them of the chemicals by airing. It appears from these tests that the chemicals either penetrate and kill the dry conidia or in some way become

TABLE 5.—*The effect of exposing air dry conidia of Glomerella gossypii on filter paper at different temperatures to vapor from 3 preparations containing organic mercury*

| Treatment | | | Number of cultures alive out of 5 treated | |
|---|---|---|---|---|
| Active ingredient in preparation used | Temperature | Time | | |
| | °C. | *Hours* | *Expt. 1*[d] | *Expt. 2*[d] |
| Control, not gassed | Room | | 5 | 5 |
| Ethyl mercury chloride, 2 per cent[a] | 5 | 2 | 0 | 0 |
| " " " " " " | " | 4 | 0 | 0 |
| " " " " " " | 28 | 2 | 0 | 0 |
| " " " " " " | " | 4 | 0 | 0 |
| Ethyl mercury phosphate, 5 per cent[b] | 5 | 2 | 2 | 2 |
| " " " " " " | " | 4 | 1 | 1 |
| " " " " " " | 28 | 2 | 0 | 0 |
| " " " " " " | " | 4 | 0 | 0 |
| | | | *Expt. 3*[d] | *Expt. 4*[d] |
| Ethanol mercury chloride, 2 per cent[c] | 18 | 72 | 5 | 5 |
| Control, not gassed | " | " | 5 | 5 |
| Ethanol mercury chloride, 2 per cent | 28 | " | 5 | 5 |
| Control, not gassed | " | " | 5 | 5 |
| Ethanol mercury chloride, 2 per cent | 38 | " | 5 | 4 |
| Control, not gassed | " | " | 5 | 5 |
| Ethyl mercury chloride, 2 per cent | 18 | " | 0 | 0 |
| " " " " " " | 28 | " | 0 | 0 |
| | | | *Expt. 5*[e] | |
| Ethanol mercury chloride, 2 per cent | 28 | 24 | 5 | |
| " " " " " " | " | 48 | 5 | |
| " " " " " " | " | 72 | 5 | |
| Control, not gassed | " | " | 5 | |

[a] Ceresan; 5 grams in each gassing dish.
[b] New Improved Ceresan; 2 grams in each dish.
[c] Sanoseed; 5 grams in each dish.
[d] Gassed in large, unsealed culture dishes.
[e] Gassed in sealed desiccator dishes.

attached to the cultures (conidia and paper) so firmly as to considerably retard release when the cultures are removed to open air.

A number of experiments were made in which conidia of *Glomerella gossypii* were put on filter-paper squares after such squares had been gassed. These experiments showed that ethyl mercury chloride and phosphate vapor condensed on the paper alone in sufficient quantity to stop growth of conidia subsequently placed on the papers and that removal of the inhibiting chemicals required a period of airing considerably longer than the period of gassing in which the chemical vapors condensed on the papers. Table 7 gives

the results of an experiment in which the effects of adding the conidia to the papers before or after gassing are compared. Two series of papers were exposed for 1 hour to vapors of ethyl mercury chloride. In series A, the conidia of *G. gossypii* were put on papers and dried. Then the gassed cultures were aired as shown. In series B, the conidia were put on the papers

TABLE 6.—*Effect of airing after exposing air dry conidia of Glomerella gossypii on filter paper to vapor emanating from preparations containing organic mercury*

| Exp. No. | Active ingredient in preparation used[a] | Period of gassing | Period of gassing | Cultures alive out of 5 tested |
|---|---|---|---|---|
| | | *Hours* | *Hours* | *Number* |
| 1 | Control, not gassed | | | 5 |
| | Ethyl mercury phosphate, 5 per cent | 24 | 0 | 0 |
| | " " " " " " | " | 24 | 0 |
| | Ethyl mercury chloride, 2 per cent | " | 0 | 0 |
| | " " " " " " | " | 24 | 0 |
| | Ethyl mercury borate, 5 per cent | " | 0 | 0 |
| | " " " " " " | " | 24 | 0 |
| | Ethyl mercury iodide, 5 per cent | " | 0 | 0 |
| | " " " " " " | " | 24 | 0 |
| 2 | Control, not gassed | | | 5 |
| | Ethyl mercury phosphate, 5 per cent | 1 | 0 | 0 |
| | " " " " " " | 1 | 24 | 5 |
| | " " " " " " | 3 | 0 | 0 |
| | " " " " " " | 3 | 24 | 0 |
| | Ethyl mercury chloride, 2 per cent | 1 | 0 | 0 |
| | " " " " " " | 1 | 24 | 1 |
| | " " " " " " | 3 | 0 | 0 |
| | " " " " " " | 3 | 24 | 0 |
| | Ethyl mercury borate, 5 per cent | 1 | 0 | 5 |
| | " " " " " " | 1 | 24 | 5 |
| | " " " " " " | 3 | 0 | 1 |
| | " " " " " " | 3 | 24 | 5 |
| | Ethyl mercury iodide, 5 per cent | 1 | 0 | 0 |
| | " " " " " " | 1 | 24 | 5 |
| | " " " " " " | 3 | 0 | 0 |
| | " " " " " " | 3 | 24 | 4 |
| 3 | Control, not gassed | | | 5 |
| | Ethyl mercury chloride, 2 per cent | 4 | 0 | 0 |
| | " " " " " " | 4 | 25 | 0 |
| | Ethyl mercury phosphate, 5 per cent | 4 | 0 | 0 |
| | " " " " " " | 4 | 25 | 0 |
| | Ethyl mercury borate, 5 per cent | 4 | 0 | 0 |
| | " " " " " " | 4 | 25 | 5 |
| | Ethyl mercury iodide, 5 per cent | 4 | 0 | 0 |
| | " " " " " " | 4 | 25 | 1 |

[a] The quantity of each preparation used was such as to give an equivalent quantity of active ingredient in each dish.

after the latter had been gassed and aired. Both series were gassed concurrently in the same chamber for 1 hour at 25° C., and then aired concurrently, in the same location, at 25° C. For each treatment in each series, 10 cultures, 5 in each of 2 Petri dishes, were tested for viability. As indicated by lot 2 (Table 7), gassing the papers before or after adding conidia prevented growth of all cultures if the gassed papers were not aired before being placed

on agar. Airing for 4 hours subsequent to gassing, enabled 1 culture of series A and 7 of series B to grow. Airing for 8 hours subsequent to gassing permitted only 2 cultures of series A to grow, while 10 grew in series B. Airing 16 or 24 hours permitted only 8 cultures of series A to grow, while 10 grew in series B. The colonies in lots 4, 5, 6 from cultures of series A were noticeably smaller than those from series B, due to a slowing up of germination by the chemical or to a reduction of the number of conidia

TABLE 7.—*Effect of airing culture papers exposed to vapors of ethyl mercury chloride before or after adding conidia of Glomerella gossypii*

| Lot No. | Treatment | | Number of cultures alive out of 5 tested | | | |
|---|---|---|---|---|---|---|
| | | Period of airing hrs. | Series A. Papers[a] gassed after adding conidia | | Series B. Papers[b] gassed before adding conidia | |
| | | | Plate 1 | Plate 2 | Plate 1 | Plate 2 |
| 1a | Not gassed | 25 | 5[l] | 5[l] | ..... | ..... |
| 1b | Not gassed | 0 | ..... | ..... | 5[l] | 5[l] |
| 2 | Gassed | 0 | 0 | 0 | 0 | 0 |
| 3 | Gassed | 4 | 0 | 1[s] | 2[s] | 5[s] |
| 4 | Gassed | 8 | 2[s] | 0 | 5[l] | 5[l] |
| 5 | Gassed | 16 | 5[s] | 3[s] | 5[l] | 5[l] |
| 6 | Gassed | 24 | 5[s] | 3[s] | 5[l] | 5[l] |

[a] Except lot 1a; [b] except lot 1b; [l] colonies large; [s] colonies small.

germinating in series A. In lot 3, aired for 4 hours, colonies were small in both series A and B. No colony size differences were evident between the controls for the two series. Since fewer cultures grew and colonies were smaller in series A, in which both paper and conidia were gassed, than in series B, in which only the paper substratum was gassed, it appears that the chemical used adheres somewhat more tenaciously to conidial walls than to the filter paper.

The foregoing tests show that vapors of the chemicals used adhere to filter paper in quantities lethal to *Glomerella gossypii* and that the chemicals adhere for considerable periods of time after exposure to vapor-free air. An effort was made to learn if treatment of conidia deposited on glass would have a different effect than treatment of conidia on paper. Conidia of *G. gossypii,* dried down on sterile filter paper and on glass cover slips, were gassed by ethyl mercury chloride and ethyl mercury phosphate in sealed desiccator dishes at 3 temperatures for 2 time intervals (Table 8). Growth of cultures on paper was inhibited at all exposure periods by both chemicals at 13° and 28° C. and also at 5° C. by ethyl mercury chloride. On glass no inhibition occurred, except by ethyl mercury chloride at 5° C. and 4 hours' exposure.

In other experiments, conidia on glass cover slips were exposed to ethyl mercury chloride and ethyl mercury phosphate for periods of 12 or more hours in sealed dishes (Table 9). In experiment 1, the conidia were dried on cover slips for a uniform period of time before gassing. In experiments

2 and 3 the conidia were dried for short and long periods of time. It was expected that differences in moisture content of the conidial walls might result from short and long drying periods and that this would in turn effect absorption of the chemical vapors and produce demonstrable differences in viability of the treated cultures. Such results did not follow. Conidia dried in experiment 3 for so short a period as 40 minutes, still were able to germinate after being gassed as long as 48 hours on glass. In other experiments noted above, exposure of conidia on filter paper for 12 or 24 hour periods to vapors of these chemicals resulted invariably in inhibited growth.

TABLE 8.—*Effect of exposing air dry conidia of Glomerella gossypii on filter paper and on glass coverslips to vapors emanating from preparations containing organic mercury compounds at different temperatures*

| Active ingredient in preparation used | Temperature during treatment[a] ° C. | Period of gassing hours | Number of cultures alive out of 5 gassed on | |
|---|---|---|---|---|
| | | | Paper | Glass |
| Control, not gassed | Room | | 5 | 5 |
| Ethyl mercury chloride, 2 per cent[b] | 5 | 2 | 0 | 5 |
| " " " " " " | 5 | 4 | 0 | 0[d] |
| " " " " " " | 13 | 2 | 0 | 5 |
| " " " " " " | 13 | 4 | 0 | 5 |
| " " " " " " | 28 | 2 | 0 | 5 |
| " " " " " " | 28 | 4 | 0 | 5 |
| Ethyl mercury phosphate, 5 per cent[c] | 5 | 2 | 5 | 5 |
| " " " " " " | 5 | 4 | 5 | 5 |
| " " " " " " | 13 | 2 | 0 | 5 |
| " " " " " " | 13 | 4 | 0 | 5 |
| " " " " " " | 28 | 2 | 0 | 5 |
| " " " " " " | 28 | 4 | 0 | 5 |

[a] Treatments were made in desiccator dishes having lids sealed with petrolatum.
[b] Ceresan; 5 g. in each dish.
[c] New Improved Ceresan; 2 g. in each dish.
[d] Microscopic examination showed many conidia on each coverglass, but none had germinated.

The results presented in tables 8 and 9, taken in conjunction with data presented in preceding tables, show that conidia on glass are less readily inhibited from growth by exposure to vapors of the chemicals used than conidia on paper. Seemingly, in the foregoing experiments the chemical vapors had as great opportunity to condense on, enter and kill dry conidia gassed on glass as when gassed on paper. Apparently failure of the paper cultures to grow was due chiefly to action of vapor condensed on and held in the paper and later absorbed by the conidia when placed on moist agar. The chemicals failed to adhere to glass in the same quantities as to paper. The experiments with seeds (Tables 1, 2, 3) indicate that cotton lint may hold the condensed chemical vapors much as does filter paper.

In preliminary tests, cultures of *Glomerella gossypii* growing on moist potato-dextrose agar in Petri dishes were exposed to vapors of ethyl mercury chloride (Ceresan) in sealed dishes. Conidia thus gassed failed to grow when transferred to fresh nutrient agar. In other tests cultures of *G.*

TABLE 9.—*Effect of exposing dry conidia of Glomerella gossypii on glass cover slips to vapors emanating from preparations containing organic mercury*

| Active ingredient in preparation used | Hours of gassing | Number of cultures alive out of 5 or 10 gassed | | |
|---|---|---|---|---|
| | | Exp. 1[a] | Exp. 2[bc] | Exp. 3[def] |
| Ethyl mercury chloride, 2 per cent | 12 | 4 | | |
| " " " " " | 24 | 3 | 5 – 10 | 5 – 5 – 5 |
| " " " " " | 48 | ... | ... ..... | 5 – 5 – 5 |
| " " " " " | 72 | ... | ... ..... | ... ... 4 |
| Ethyl mercury phosphate, 5 per cent | 12 | 4 | ... ..... | ... ... ... |
| " " " " " | 24 | 5 | 10 – 10 | ... ... ... |
| Control[g] | 0 | 5 | 10 – 10 | 5 – 5 – 5 |

[a] Conidia dried on cover slips 7 or 8 days before gassing. Five cultures gassed for each treatment.

[b-c], Conidia of [b] and [c] from same culture, gassed concurrently in same gassing chamber: [b] dried 4 hr., [c] dried 28 hr. before gassing. Ten cultures gassed each treatment combination.

[d-e, f]. Conidia of [d] dried on cover slips 40 minutes (until all macroscopically visible water had evaporated), [e] dried 1 hr. 40 min., [f] dried 24 hr. before gassing. Five cultures gassed for each treatment combination.

[g] Not gassed; stored in empty dish until end of longest gassing period used in the experiment.

*gossypii* were allowed to dry in a drying cabinet for various periods of time before exposure to vapors of the chemical. At the end of the gassing period, conidia, and in some tests conidia and pieces of agar substratum, were removed to nutrient agar. Details and results of some of these tests are given in table 10. The gassed conidia failed to grow, even though the gassing was done after the cultures had been permitted to become completely air dry. Enough of the chemical was taken up from the vapors and retained by the air-dry conidia alone to prevent subsequent growth.

TABLE 10.—*Effect of vapors of ethyl mercury chloride on conidia of Glomerella gossypii growing on nutrient agar in Petri dishes*

| Exp. No. | Treatment of fungus | Estimated percentage water in substratum | Fungus alive in 10 transfers of | |
|---|---|---|---|---|
| | | | Conidia only | Substratum and conidia |
| 1 | Not dried, gassed | 95 | 2 | 0 |
| | Dried 24 hr., gassed | 70 | 0 | 0 |
| | Dried 48 hr., gassed | 20[a] | 0 | 0 |
| | Dried 66 hr., gassed | 15[a] | 0 | 0 |
| | Dried 48 hr., not gassed | 20[a] | 10 | 10 |
| 2 | Dried 55 hr., gassed | 15[a] | 0 | |
| | Dried 55 hr., not gassed | 15[a] | 10 | |
| 3 | Dried 66 hr., gassed | [b] | 0 | |
| | Dried 66 hr., not gassed | [b] | 10 | |
| 4 | Not dried, gassed | 95 | 0 | 0 |
| | Dried 72 hr., gassed | [b] | 0 | 0 |
| | Dried 72 hr., not gassed | [b] | 10 | 10 |

[a] Not completely air dry; cuts like rubber, but readily removed from dish.
[b] Completely air dry, difficult to remove pieces of substratum from dish.

### TESTS WITH OTHER CHEMICALS

In the course of the work herein reported, conidia on filter paper squares were exposed to vapors that might arise from a number of chemicals other than those discussed above. The results from the tests with these preparations are given in table 11. Little or no vapor action was found for Semesan, creosote dust, benzol, benzine, paradichlorobenzene, or picric acid. Vapors from alkyl mercuric acetylene were partly effective in killing the conidia. This effectiveness is considerably enhanced by addition of urea. Leytosan, a preparation containing mercury in combination with phenol and urea, was slightly less effective than alkyl mercuric acetylene plus urea. A high proportion of the conidia were killed by vapors of 154–6B and trioxymethane. The latter did not readily evaporate from the filter paper that carried the conidia and probably would act in a similar manner on the short fibers on cotton seed. Vapor of chloropicrin killed all cultures gassed for 72 hours but only part of those gassed 24 hours. Ethyl ether was not effective at 24 hours' exposure, but prevented growth of all cultures gassed for 72 hours.

TABLE 11.—*Effect of exposing air-dry conidia of Glomerella gossypii on filter paper to vapors emanating from various chemical preparations*

| Preparation and/or active ingredient therein | Period of gassing hours | Number cultures alive/number treated | | |
|---|---|---|---|---|
| | | Control, cultures not gassed | Cultures gassed | |
| | | | Not aired after gassing | Aired after gassing |
| Semesan (hydroxymercurochlorophenol), 30 per cent | 24 | 10/10 | 10/10 | |
| " " " | 72 | 5/5 | 5/5 | |
| Alkyl mercuric acetylene[a] | 24 | 10/10 | 8/10 | |
| " " " | 72 | 10/10 | 5/10 | |
| Alkyl mercuric acetylene plus urea[b] | 24 | 5/5 | 0/5 | |
| " " " " | 72 | 10/5 | 0/10 | |
| 154–6B[d] | 28 | 10/10 | 0/10 | |
| Leytosan (Phenolmercuricurea) | 24 | 10/10 | 3/10 | |
| " " | 72 | 10/10 | 0/10 | |
| Creosote dust (5 per cent creosote[c]) | 24 | 15/15 | 15/15 | |
| " " " " " | 72 | 5/5 | 5/5 | |
| Chloropicrin | 24 | 10/10 | 6/10 | |
| " | 72 | 5/5 | 0/10 | |
| Benzol C.P. | 24 | 10/10 | 10/10 | |
| " " | 72 | 5/5 | 5/5 | |
| Benzine (Petroleum ether) C.P. | 24 | 10/10 | 6/10 | |
| " " " " | 72 | 5/5 | 5/5 | |
| Ethyl ether C.P. | 24 | 10/10 | 10/10 | |
| " " " | 72 | 5/5 | 0/5 | |
| Para Bacca (Paradichlorobenzene) | 24 | 10/10 | 10/10 | |
| " " " | 72 | 5/5 | 5/5 | |
| Trioxymethane U.S.P. | 24 | 10/10 | 2/10 | 0.5 |
| " " | 72 | 5/5 | 0/5 | |
| Picrin acid crystals | 24 | 5/5 | 5/5 | |
| " " " | 72 | 5/5 | 5/5 | |

[a] "M" compound, supplied by Chicago Development Laboratory, described as "insoluble."

[b] Supplied by Chicago Development Laboratory, described as "soluble."

[c] Supplied by Heyden Chemical Corporation, New York.

[d] Supplied by American Cyanamid and Chemical Corporation.

## DISCUSSION

The experiments described in this paper show that cotton seeds infested with certain fungi are greatly benefited by confinement for a time in an atmosphere containing vapors emanating from certain organic mercury preparations. Likewise, conidia of *Glomerella gossypii* on filter paper were prevented from germination by suitable exposure to vapor from these materials. Preparations containing ethyl mercury chloride and ethyl mercury phosphate were much more effective in vapor action than similar preparations containing ethanol mercury chloride, and hydroxymercurochlorophenol, while ethyl mercury borate was intermediate in effectiveness. This difference appears to be correlated with volatility of the active materials. Ethyl mercury chloride and ethyl mercury phosphate are characterized by considerable volatility, while ethanol mercury chloride and hydroxymercurochlorophenol are relatively non-volatile at ordinary temperatures, and ethyl mercury borate is intermediate in this respect. In field tests with cotton seed dusted with preparations of ethanol mercury chloride and hydroxymercurochlorophenol, these 2 relatively non-volatile chemicals usually have been less beneficial than preparations containing the more volatile salts, ethyl mercury chloride or ethyl mercury phosphate.

The adherence of vapors of ethyl mercury chloride and ethyl mercury phosphate to filter paper and to conidia after removal from the gassing chambers to open air may be due to some physical bond with the substance of the paper or conidial walls or to diffusion into and retention by submicroscopic pores in the paper and conidial walls. After a time the vapors escape, thus removing inhibition to germination of the conidia; but the time required for release of the chemicals is considerably longer than the time for fixation of the vapors in amounts necessary to stop germination. After longer periods of gassing, longer periods of airing were required to release the vapors and permit germination of the conidia, indicating more complete saturation of the paper and conidia. Apparently storage of seed for several days after dusting with these materials should increase the effectiveness of the treatment. Seeds that have escaped direct particle contact with the chemical applied to a given seed lot, are effectively fumigated by vapor from particles on adjacent seeds. The vapor becomes fixed to the lint and to the fungi on the seed in such fashion as to be retained for a considerable period of time after removal from confinement.

Wolf and his coworkers (13) state that vapors of benzol and paradichlorobenzene effect control of the downy mildew fungus, *Peronospora tabacina,* in tobacco beds, by solution in the water on and within plant tissues and in constituents of the living cells of the tobacco plant and of the mycelium of the fungus. In certain of the experiments made in connection with work reported in this paper, it was observed that growth of the cultures gassed with ethyl mercury chloride after putting the conidia on the paper squares was more readily prevented than when the papers were gassed before adding the conidia. Thus it seemed that during the gassing period the vapors may have entered through the dry walls of the conidia in suffi-

cient quantity to inactivate their protoplasts. Since many of such cultures grew, however, after removal to open air for a suitable period of time, it appears that the chemical vapors were deposited on but did not enter the conidia until they were placed on moist agar for germination. The technique employed in these tests is not adequate to determine whether or not some of the conidia may have been killed by penetration of the dry walls by the chemicals during the gassing period.

Concentration of vapors in a confined atmosphere above exposed surfaces is dependent on temperature. Vapor action, inhibitory of spore germination, was observed for ethyl mercury chloride and ethyl mercury phosphate at temperatures as low as 5° C. and as high as 28° C. This well covers the range of weather temperature that would exist between the usual times of treating and planting cotton seed. Efficient vapor action was not observed for ethanol mercury chloride at any temperature tried. This may be basic to the somewhat poorer results obtained from ethanol mercury chloride in field tests in which this material has been compared with ethyl mercury chloride and ethyl mercury phosphate.

In the foregoing tests it has been assumed that the lethal vapors were those of the substances reported by the manufacturer to be the principal active ingredient in the preparations tested. However, since the mercury preparations used in most of the experiments were of commercial manufacture and were not made by mixing chemically pure salts with the diluents, it is recognized that minor percentages of other mercury compounds may have been present.

## SUMMARY

The commercial seed treatment preparations sold as Ceresan and New Improved Ceresan and containing ethyl mercury chloride and ethyl mercury phosphate, respectively, as the chief active ingredient emit fungicidally active vapor. When cotton seeds were confined with this vapor, the vapor condensed on the seeds in sufficient quantity to inhibit growth of seed-infesting fungi. Similarly, vapor from preparations containing ethyl mercury borate and ethyl mercury iodide were found to act as a fumigant fungicide for cotton seeds.

Mercurial vapor from the 4 preparations mentioned above was lethal to conidia of *Glomerella gossypii* exposed on filter paper and on mycelium in Petri dishes. The vapor was retained by the paper and the conidia in quantity lethal to the conidia for considerable periods of time after removal to open air. The chemical appeared to be retained more tenaciously by conidia than by filter paper. In order of decreasing effectiveness of their vapor action against fungi on seeds and filter paper, these 4 chemicals rank as follows: ethyl mercury chloride, ethyl mercury iodide, ethyl mercury phosphate, and ethyl mercury borate, respectively.

Sanoseed, containing ethanol mercury chloride, gave good control of seed-infesting fungi when applied directly to cotton seed, but little or no beneficial effect was found when infested seed or conidia of *G. gossypii* on filter paper were exposed to its vapor at temperatures of 38° C. or lower.

Ethanol mercury chloride is of low volatility at temperatures such as those to which treated cotton seed would be exposed.

By reason of the volatile nature of ethyl mercury chloride and ethyl mercury phosphate and their property of condensing on and adhering to cotton seed and seed-infesting fungi, very small ratios of quantity of chemical to quantity of seed are effective. Vapor from seeds carrying bits of the chemical pass to and condense on adjacent seeds that may have received none of the chemical in the dusting operation. Vapor action of ethyl mercury chloride and ethyl mercury phosphate is effective in the range of temperatures (5° to 38° C.) likely to obtain between the dates of treating and planting cotton seed.

Tests of Semesan (30 per cent hydroxymercuro-chlorophenol), creosote dust (5 per cent creosote), benzol, benzine (petroleum ether), para bacca (paradichlorobenzene), and picric acid, showed no inhibitory effect of their vapors toward air-dry conidia of *G. gossypii*. Marked reduction or complete prevention of germination of conidia of this fungus was effected by vapors of alkylmercuricacetyleneurea, leytosan (phenomercuricurea), 154–6B, chloropicrin, ethyl ether, and trioxymethane. Alkylmercuricacetylene was only partly effective as a fumigant fungicide.

STATE COLLEGE OF AGRICULTURE AND ENGINEERING,
RALEIGH, NORTH CAROLINA.

LITERATURE CITED

1. ARNDT, C. H. A résumé of cotton seed treatment tests in South Carolina. Phytopath. 25: 970. 1935.
2. BROWN, CECIL C. Seed disinfection by formaldehyde vapor. Jour. Agr. Res. [U.S.] 17: 33–39. 1919.
3. GIESE, ARTHUR C. Mercury poisoning. Science 91: 476–477. 1940.
4. HANCOCK, N. I., and D. M. SIMPSON. Cotton seed treatments in Tennessee. Tenn. Agr. Exp. Stat. Bull. 175. 1941.
5. KENKNIGHT, GLENN. Studies on soil actinomycetes in relation to potato scab and its control. Mich. Agr. Exp. Stat. Tech. Bull. 178. 1941.
6. KINCAID, RANDALL R. Toxicity of mercury vapor to germinating tobacco seeds. Pl. Phys. 11: 654–656. 1936.
7. LEHMAN, S. G. Seedling survival as affected by certain mercury, copper, and zinc preparations not included in regional seed treatment tests for 1938. Proc. Asso. Sou. Agr. Workers, 40th Ann. Con., New Orleans, 1939, p. 188.
8. LEUKEL, R. W. Seed treatment for control of covered smut of barley. U. S. Dept. Agr. Tech. Bull. 207. 1930.
9. ————————. Studies on bunt or stinking smut of wheat, and its control. U. S. Dept. Agr. Tech. Bull. 582. 1937.
10. ————————. Seed treatment experiments with oats naturally and artificially inoculated with smuts. U. S. Dept. Agr. Tech. Bull. 568. 1937.
11. MILES, L. E. Effect of type and period of storage on cotton seed after treatment with organic mercury dusts. Phytopath. 29: 986–991. 1939.
12. SMITH, H. P., D. L. JONES, D. T. HILLOUGH, H. C. MCNAMARA. Chemical dust treatment of cotton seed for planting purposes. Texas Agr. Exp. Stat. Bull. 531. 1936.
13. WOLF, FREDERICK A., RUTH A. MCLEAN, J. A. PINCKARD, F. R. DARKIS, and P. M. MOSS. Volatile fungicides, benzol and related compounds, and the principles involved in their use. Phytopath. 30: 213–227. 1940.
14. WOODRUFF, NAOMI C. Treating cotton seed by the dusting method. Ga. Agr. Exp. Stat. Bull. 170. 1931.
15. YOUNG, V. H., and C. K. MCCLELLAND. Seed treatments for corn, oats and barley in Arkansas. Ark. Agr. Exp. Stat. Bull. 389. 1940.
16. ZIMMERMAN, P. W., and WILLIAM CROCKER. The injurious effect of mercury vapor from bichloride of mercury in soil of rose houses. Florists Exch. and Hort. Trade World 81: 222–225. 1933.

# A DESTRUCTIVE VIRUS DISEASE OF SOUR CHERRY[1]

G. W. KEITT AND C. N. CLAYTON

(Accepted for publication September 29, 1942)

For many years an unfruitful condition of sour cherry (*Prunus cerasus* L.), known to the growers as "yellow leaf" or "boarder tree," has been observed in Wisconsin. Similar or identical troubles have been reported by Stewart (21) and Gloyer and Glasgow (6) as a non-parasitic "yellow leaf" and by Rasmussen (17) as "physiological yellow leaf." Various possible causes of the trouble have been suggested, but convincing experimental evidence as to cause has been lacking. Observations that the malady was spreading in Wisconsin orchards led the writers in 1936 to underake studies that have shown that they are dealing with a virus disease, or possibly a complex of virus diseases. Brief reports on this work have been published (3, 4, 11, 12, 13, 14, 15). The present paper[2] reports the progress of this investigation from its beginning to the end of the season of 1940.

Cherry yellows is tentatively proposed (*cf.* 4) as the common name of this disease because of the type of leaf symptoms and the probability discussed herein that it is transmitted by leaf hoppers.

In the present state of knowledge of virus diseases of stone fruits, it is not possible to give a complete account of the etiology and symptom expression of cherry yellows or to define its relations to certain other virus diseases. The following account is to be regarded as a report of progress based chiefly on field experiments and observations extending over a 5-year period. In some respects, especially the identity of the virus or virus complex and the range of symptom expression, it is subject to modification or correction in the light of results of further studies now in progress.

A general review of literature relating to virus diseases of stone fruits seems unwarranted here, since, for present purposes, the field has been sufficiently covered by others (1, 2, 9).

Since this paper was drafted, several articles on the disease under consideration have appeared (9, 10, 18, 19). These will be referred to later in connection with topics on which they bear.

## SYMPTOMS

The leaf symptoms described below are characteristic of the disease as the writers have seen it develop naturally or after transmission by budding in the field.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] This paper was prepared in the summer of 1940, when one of the writers was called to another post. It was held in the hope that the results of 1941 would yield conclusive evidence regarding insect transmission and provide some quantitative data on the influence of the disease on fruitfulness of the trees. The leaf symptom expression of the disease in Wisconsin was so poor in 1941 that results from the transmission experiments could not be taken in that season. The paper, with minor revision, is therefore being published,

Chlorotic areas of lighter than normal green appear on any part of the leaf lamina. They are very variable in size and shape, sometimes showing in this early stage a more or less definite ring-spot effect. If the leaves are not abscised at an early stage of symptom expression, the chlorotic areas become progressively paler green and then progressively yellow to the shade

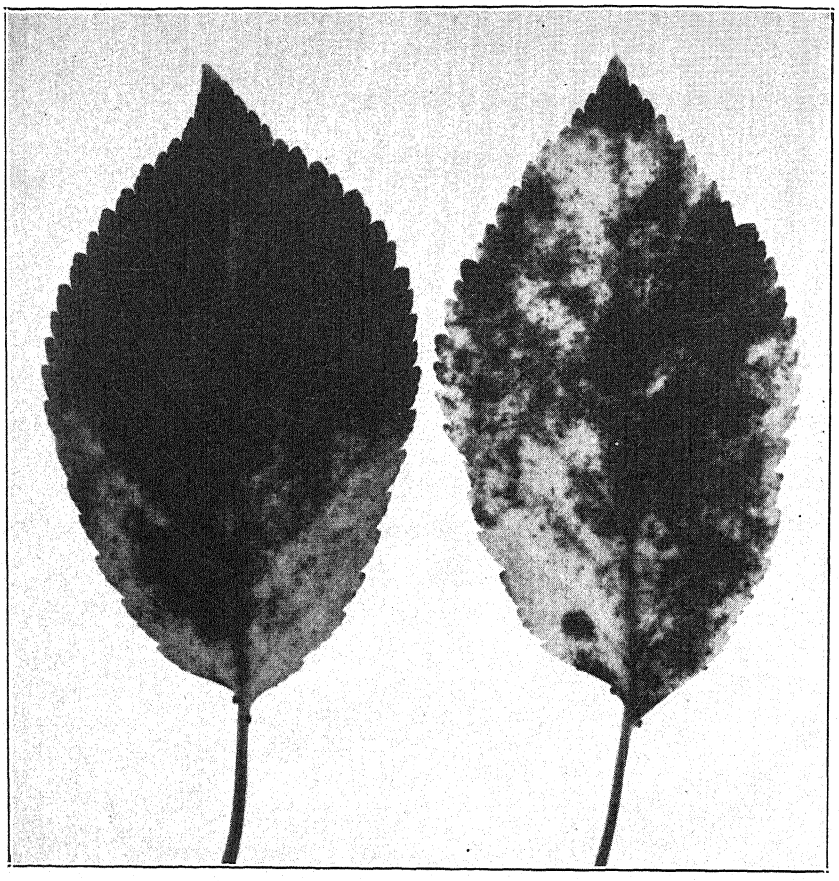


FIG. 1. Montmorency leaves showing symptoms of yellows. Photographed June 29, 1939.

characteristic of yellows leaves in the more advanced stages of symptom development. The range of yellow color is chiefly between the shades of Martius and Jasmine, as shown in the dictionary of color by Maerz and Paul (16). The affected leaves then exhibit a very conspicuous mottling of yellow and green (Fig. 1), and if they persist long enough on the tree, some of them may become entirely yellow. The green color may persist longest near the midrib and the main veins. Sometimes, however, this rela-

and it is contemplated that the results of work after 1940 will be treated in later publications.

Acknowledgments are made to J. D. Moore for assistance in the work done in 1940 and 1941; to Eugene Herrling for the photographic work.

tion does not obtain. In some cases the green fades and the yellow comes in rather uniformly over a large part of the leaf. Small islands of darker green may be left scattered in such areas. There is usually no necrosis, except occasionally at the tip of the leaf. These mottled or yellow leaves and some that are still green are soon abscised.

The leaf symptoms usually begin to appear about 3 or 4 weeks after petal-fall. A major wave of defoliation ordinarily begins in the Door County district in late June or early July and continues for about 2 or 3 weeks. A few affected leaves or minor waves of defoliation may be observed for some weeks later. The defoliation typically begins with the older leaves, commonly including the scale leaves, and extends towards the younger. Often, however, a leaf at or near the apex of a terminal shoot will show the symptoms in the latter part of the season, while the older leaves that remain on the shoot do not. The defoliation usually includes about 1 to 50 per cent of the leaves. Some of the leaves of diseased trees may be larger than normal, and the foliage of badly diseased trees, when viewed from a distance of about 10 to 50 feet, tends to appear lighter green than normal.

The disease does not kill the trees outright, but progressively and at variable rates over a period of years it impairs their health and fruitfulness, and probably tends to shorten their lives.

A fully authenticated description of the progressive development of the disease must await the results of extensive studies in which many cases are followed, with appropriate experimentation, throughout their complete histories. The following statements, which are subject to revision when more complete information is available, represent the writers' present judgment regarding certain aspects of the development of the disease as they have seen it in Wisconsin orchards.

The disease does not commonly seem seriously to impair the fruitfulness of the tree in the first year of symptom expression. Usually a few yellow leaves appear in the first year the disease is observed. By the second or third year defoliation may be severe and the crop much impaired. The rate of progressive development of the disease varies much with factors not yet

TABLE 1.—*Relation of cherry yellows to yield, Montmorency variety, Sturgeon Bay, Wis., 1941*

| Year tree first mapped as diseased | Pairs[a] of trees | Average yield per diseased tree | Average yield per healthy tree | Indicated average reduction in yield due to virus | |
|---|---|---|---|---|---|
| | *Number* | *Pounds* | *Pounds* | *Pounds* | *Per cent* |
| 1936 | 18 | 81.3 | 139.1 | 57.8 | 41.6 |
| 1937 | 6 | 71.0 | 125.8 | 54.8 | 43.6 |
| 1938 | 8 | 129.6 | 157.9 | 28.3 | 17.9 |
| 1939 | 14 | 107.9 | 129.6 | 21.7 | 16.7 |
| 1940 | 4 | 131.0 | 123.8 | ..... | ..... |

[a] Diseased trees were chosen from a survey map of the orchard without observation of the trees, and each was paired with an adjacent healthy tree selected at random from this map.

clearly understood. Among possible factors governing this rate of development are virus strains or combinations, variations in the hereditary resistance of host strains or varieties, and the play of varying environmental conditions.

Limited evidence of a progressive effect of the disease on fruitfulness of the trees is shown in data taken in 1941 (Table 1). There is no evidence of

FIG. 2. Fruiting branches from the more vigorous growth of diseased (left) and healthy (right) Montmorency trees. The diseased tree is known to have shown symptoms of yellows since 1936. Strings and arrows mark the annual linear growth. The scale shown is in inches. Photographed July 19, 1941. There was very little expression of the leaf symptoms of yellows in Wisconsin in 1941.

reduction in yield of the trees on which the symptoms of the disease were first observed in 1940. The reduction appears to have been moderate on trees known to have shown symptoms for 2 or 3 years and severe on trees known to have shown symptoms for 4 years or more. These statements, it should be noted, are based on averages from comparatively small numbers

of trees. It is planned that the detailed data of table 1, with statistical treatment, will be reported in a later publication with further data from the same and other trees. More extensive data on the comparative yield of diseased and healthy trees, without record of the length of time the diseased trees had shown symptoms, are given by Rasmussen and Cation (18). The average yield of the diseased trees reported by these investigators was approximately 47 per cent that of the healthy trees.

A detailed account of the effects of the disease on the growth and fruitfulness of the tree must await the results of further work. It may be said now that the spur system of the diseased tree tends progressively to become

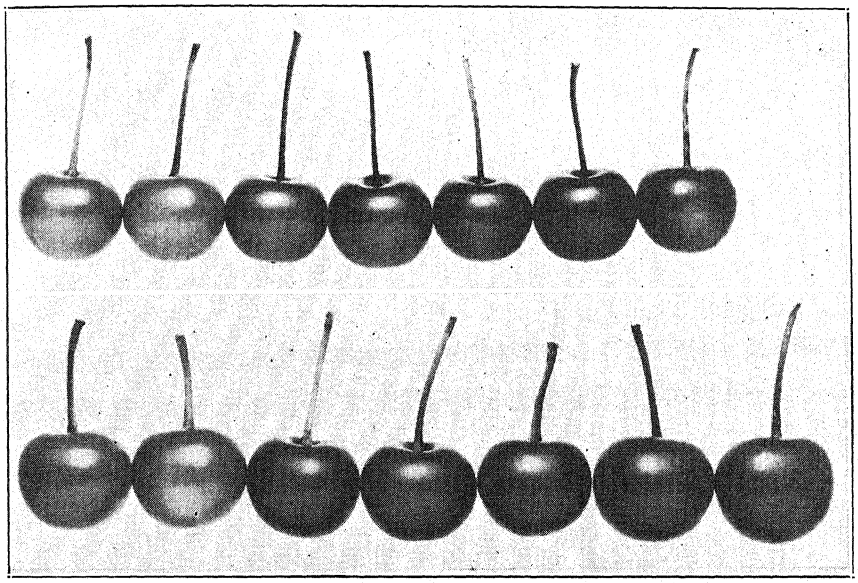


FIG. 3. Montmorency fruits from healthy (top row) and diseased (bottom row) trees.

much reduced over a period of years (*cf.* 17), in comparison with that of a healthy tree (Fig. 2).

The fruits from diseased trees are of good quality and free from any external or internal necrosis or bumpiness. They tend to be larger (often as much as 20 or 30 per cent) than average-size fruits from healthy trees (Fig. 3). Samples of 500 fruits, 10 fruits taken at random from each of 50 diseased trees, and similar samples from each of 50 adjacent healthy trees were collected on 2 dates in each of the years 1936–1939, inclusive. The average weight of the samples from the diseased trees was greater than from healthy trees in the stated years by the following percentages: 1936, 16; 1937, 32; 1938, 17; and 1939, 30. It is probable that the large size of the fruit is correlated in some degree with the sparseness of the crop (*cf.* 11). Brix tests in 1937 showed that sap of fruits from diseased and healthy trees, respectively, had about the same sugar content. The fruit symptoms of this

disease are in marked contrast to those described by Atanasoff (1) and Christoff (2) for mosaic of stone fruits in Europe and those of the pink fruit disease of sour cherry reported by Heald, Jones, and Huber (7) and Reeves, Huber, and Baur (20).

Diseased trees tend to bloom about 2 days later than healthy ones. The petals on some of their blossoms may have pink lines or splotches. The blossom buds of diseased trees have at times seemed to be more subject to winter injury or frost injury than those of healthy trees.

Symptoms on Montmorency and Early Richmond, the chief sour cherry varieties dealt with in this investigation, have seemed indistinguishable.

It is recognized that some of the symptoms described above may be atributable to a virus complex rather than a single virus, and that symptoms in addition to those described may be attributable to the yellows virus. In the latter regard it is noteworthy that, in greenhouse experiments that will be reported in a later paper, necrotic ring spot (9, p. 62–63) has commonly developed after chip budding from sour-cherry-yellows trees to presumably healthy sour cherry trees. A like result was reported earlier in correspondence with the writers by E. M. Hildebrand, who had budded in the greenhouse from yellows twigs procured from them. In extensive experiments in the greenhouse at Wisconsin both necrotic ring spot and a chlorotic ring spot have commonly appeared after chip budding from sour-cherry-yellows trees to presumably healthy sour-cherry trees and several other *Prunus* species. The necrotic ring spot is very prevalent in the orchards from which the yellows twigs were taken, occurring both on trees that have shown yellows symptoms and those on which yellows has not been observed. The chlorotic ring spot has been observed less frequently in the orchard but has commonly occurred in the greenhouse experiments on the same trees affected by necrotic ring spot. A rosette condition also has been encountered on sour cherry, in both orchard and greenhouse. The relations of yellows, necrotic ring spot, chlorotic ring spot, and rosette have not yet been satisfactorily clarified.

## EXPERIMENTS ON THE CAUSE OF THE DISEASE

Microscopic examinations and other laboratory studies of the diseased tissues showed no evidence that a fungus or bacterium has a causal relation to the disease.

Various fertilizer treatments were started early in the course of this investigation in order to determine whether the "yellow leaf" condition was the result of a deficiency of some necessary element or whether addition of some element or elements might affect the course of the disease or the expression of symptoms. Ferrous sulphate, zinc sulphate, manganese sulphate, borax, ammonium sulphate, potassium chloride, and calcium superphosphate were used alone and in various combinations in relatively heavy applications to the soil under diseased and healthy trees in the fall of 1937 and the spring of 1939. Injections in the trunks of diseased trees were made in 1937 with

solutions of each ferrous sulphate, manganese sulphate, and zinc sulphate. Several diseased trees were severely pruned in 1938. Visual examinations of the experimental trees in 1938, 1939, and 1940 showed that none of the diseased trees had been cured. Additional experiments with fertilizing, mulching, and pruning were started in 1940 to discover further evidence regarding the extent to which the course of development of the disease and yield of the affected trees might be modified.

### Transmission from Sour Cherry to Sour Cherry by Budding

The varieties used were Montmorency and Early Richmond. The results from these were indistinguishable and are, therefore, not listed separately.

*Experiments of 1937–1938.* Preliminary experiments were initiated in 1937 to determine whether the disease is bud-transmissible. The results in 1938, though inconclusive, strongly suggested that the disease had been transmitted.

*Experiments of 1938–1939.* In 1938, reciprocal budding experiments with diseased and healthy trees were started in 3 orchards. The budding treatments, number of trees used, and results in 1939 are shown in table 2.

TABLE 2.—*Summary of data on transmission of cherry yellows by budding, Sturgeon Bay, Wis., 1938–1939*

| Budding of trees in 1938 | | Results in 1939 | | | | |
|---|---|---|---|---|---|---|
| Treatment | Treated or control trees | Shoots developed from inserted buds | | Condition of trees | | |
| | | Healthy | Diseased | Healthy | Diseased | Doubtful |
| | *Number* | *Number* | *Number* | *Number* | *Number* | *Number* |
| Buds from diseased trees[a] inserted in healthy trees | 24 | 0 | 3 | 0 | 20 | 4[b] |
| Buds from healthy trees inserted in diseased trees | 8 | 0 | 4 | 0 | 8 | 0 |
| Controls: healthy trees not budded | 13 | ... | ... | 13 | 0 | 0 |
| Control: buds from healthy trees inserted in healthy tree | 1 | 0 | 0 | 1 | 0 | 0 |

[a] Buds were inserted from 10 diseased trees, and the disease was transmitted from each of these trees.

[b] All these trees showed typical leaf symptoms of the disease in 1940.

The details are omitted, except for the chief experiment, which is briefly described as follows:

Twenty-eight 3-year-old Montmorency trees planted 6 feet apart in a row were used. Symptoms of the disease appeared on 1 of these trees in 1936, before the experiment was started, and each year thereafter.

One or more buds from diseased trees were inserted in each of 15 apparently healthy[3] trees in late July and early August, 1938. None of the buds

[3] Trees are hereinafter referred to as healthy when they showed no symptoms of the disease at the time of observation.

from diseased trees put out shoots, although union of tissues between the inserted bud-piece and the budded tree occurred in many cases. In 1939, 11 of these 15 budded trees showed definite leaf symptoms of the disease (Fig. 4). These leaf symptoms were more pronounced on the branches close to the place of bud insertion; however, some diseased leaves were found on all branches of the trees. Doubtful symptoms occurred on the remaining 4 budded trees. In each of these cases it was doubtful whether there had been union of tissues of bud-piece and branch. All of these 4 trees, without further treatment, showed typical symptoms in 1940.

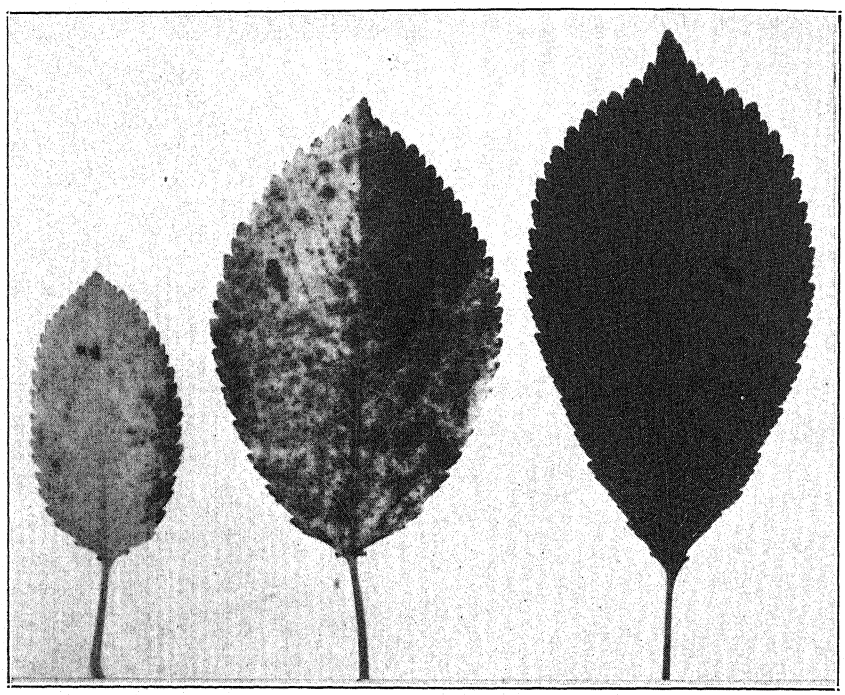


FIG. 4. Symptoms of yellows on leaves taken in serial order from the base of a shoot on a Montmorency tree to which the disease had been transmitted by budding in 1938. Only the 2 basal leaves of this shoot showed chlorosis when photographed on June 28, 1939. Two necrotic lesions, incited by *Coccomyces hiemalis* Hig., are seen on the first and third leaves.

A bud from a healthy tree was inserted in the tree (mentioned above) on which the disease had appeared in 1936. In 1939, the shoot from the inserted bud bore leaves showing the typical symptoms of the disease.

Twelve trees were left unbudded for controls. None of these showed symptoms of the disease in 1939.

*Experiments of 1939–1940.* The results of the budding experiments of 1939 are summarized in table 3. The details are omitted, except for the chief experiment, which is briefly described as follows:

Sixty-nine young Montmorency trees planted in 1939 at 2-foot intervals in rows 20 feet apart were used in the experiments on transmission of the disease by budding and by leaf hoppers. One of these trees showed the symptoms of the disease in 1939 before the experiments were started, and 2 others were doubtful.

Buds from diseased Montmorency and Early Richmond trees were inserted in 27 healthy trees and 1 that was doubtful. Twenty-six of these trees showed the characteristic leaf symptoms in 1940, and 2 were doubtful. In all cases in which union was observed between the tissues of bud-piece and

TABLE 3.—*Summary of data on transmission of cherry yellows by budding, Sturgeon Bay, Wis., 1939–1940*

| Budding of trees in 1939 | | Results in 1940 | | | | |
|---|---|---|---|---|---|---|
| Treatment | Treated or control trees | Shoots developed from inserted buds | | Condition of trees | | |
| | | Healthy | Diseased | Healthy | Diseased | Doubtful |
| | *Number* | *Number* | *Number* | *Number* | *Number* | *Number* |
| Buds from diseased[a] trees inserted in healthy trees | 52 | 0 | 1 | 6 | 40 | 6 |
| Buds from healthy trees inserted in diseased trees | 6 | 0 | 3 | 0 | 6 | 0 |
| Controls: healthy trees not budded | 32 | ... | ... | 26 | 0 | 6[b] |
| Controls: buds from healthy trees inserted in healthy trees | 18 | 17 | 0 | 16 | 2 | 0 |

[a] Buds were inserted from 17 diseased trees and the disease was transmitted from each of these trees.

[b] Observations were complicated by occurrence of leaf spot.

tree the disease was transmitted. One of the diseased buds put forth a shoot, which was diseased.

A bud from a healthy tree was inserted in the tree that was diseased when the experiment was begun. The bud put out a shoot that showed the typical leaf symptoms in 1940.

Buds from healthy trees were inserted in 2 healthy trees. One bud put out a shoot, which was healthy. Both trees were healthy in 1940.

Twenty-two healthy trees were left unbudded as controls. An outbreak of leaf spot complicated the observation of results in 1940. Sixteen of these control trees showed no evidence of the symptoms of the virus disease. Six were noted as doubtful but were thought much more likely to be free of the disease than to have it.

It is noteworthy that the experiments summarized in table 3 included transmission of the disease from 8 trees to which it had been transmitted by budding in 1938. In 1939, buds from such trees were inserted in 22 healthy trees, all of which showed typical symptoms of the disease in 1940.

*Discussion.* The foregoing experiments show that the disease on sour cherry is readily transmissible by budding. Diseased buds inserted in healthy trees usually failed to put out shoots. This was doubtless due in large measure to the fact that blossom buds were much more prevalent than leaf buds on the bud wood used. However, some union of tissues of bud-piece and the budded tree commonly occurred, and in all such cases observed the disease was transmitted. Transmission of the disease occurred in some cases in which it was uncertain whether there had been such union of tissues.

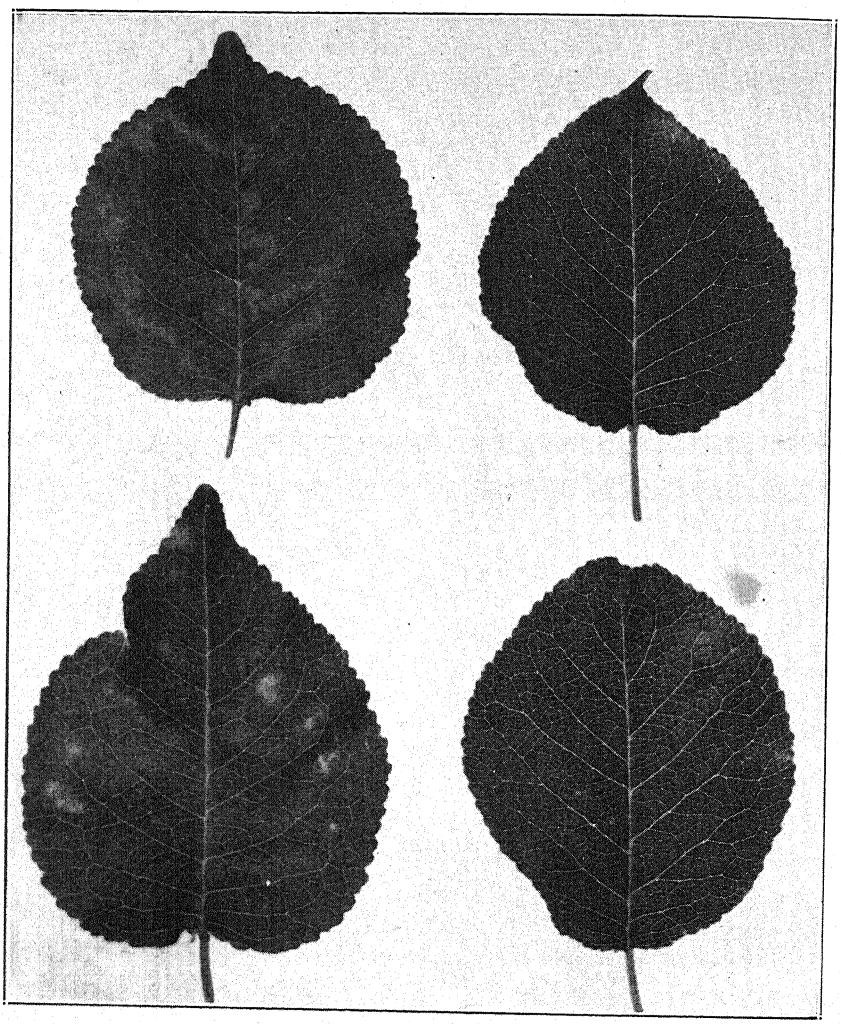


FIG. 5. Leaf symptoms on mahaleb to which the disease had been transmitted by budding from sour cherry. Left, diseased leaves from budded trees. Right, healthy leaves from controls.

## Conclusions

From the lack of association of a bacterium or a fungus with the disease, the negative results from treatments with minor elements or fertilizers, the

results of the budding experiments, and the type of symptoms, it is concluded that the inciting cause of the disease is a virus or viruses.

### PRELIMINARY EXPERIMENTS ON HOST RANGE

When, in the summer of 1939, it became clearly apparent that the disease is bud-transmissible and evidently incited by a virus or viruses, preliminary experiments on host range were initiated. Special interest attached to the 2

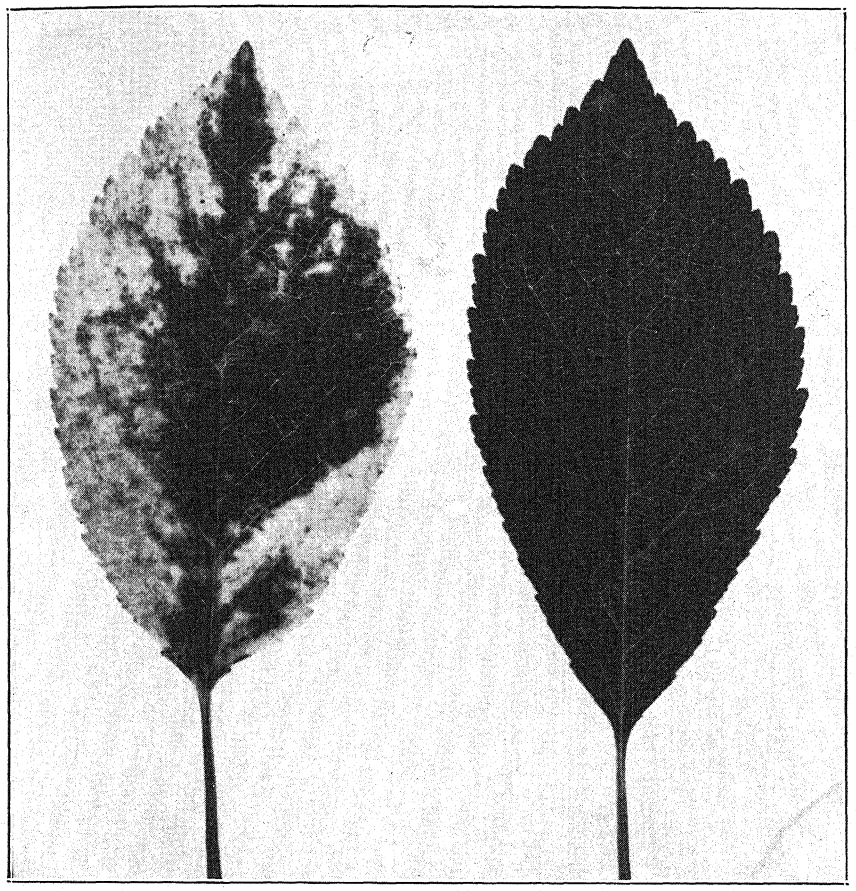


FIG. 6. Leaves from a large Montmorency tree to which the disease had been transmitted by budding from a mahaleb that had been budded from diseased sour cherry. Left, leaf taken near place of budding, showing typical symptoms. Right, leaf from opposite side of the tree, showing no symptoms. (The lighter areas represent spray residue.)

stocks on which sour cherry is commonly propagated, mahaleb (*Prunus mahaleb* L.) and mazzard (*P. avium* L.), and to other wild or cultivated species of *Prunus* that occur in the Door County section. It was necessary to use such plants as were available. The data thus far obtained are in part fragmentary, but in view of the importance of the disease it seems desirable

to report them briefly, rather than to await the results of more extensive studies.

*Prunus mahaleb.* In 1937, before transmissibility of the disease had been established, buds from diseased sour cherry were inserted in 5 healthy mahaleb sprouts. Two of these sprouts grew from the stocks of healthy sour cherry trees, 3 from the stocks of sour cherry trees that had died. In 1938, 1939, and 1940, chlorotic areas developed in some leaves of all of these budded sprouts (Fig. 5). Several similar untreated plants did not develop such symptoms. Similar symptoms have been observed on leaves of mahaleb sprouts from the stocks of diseased sour cherry trees.

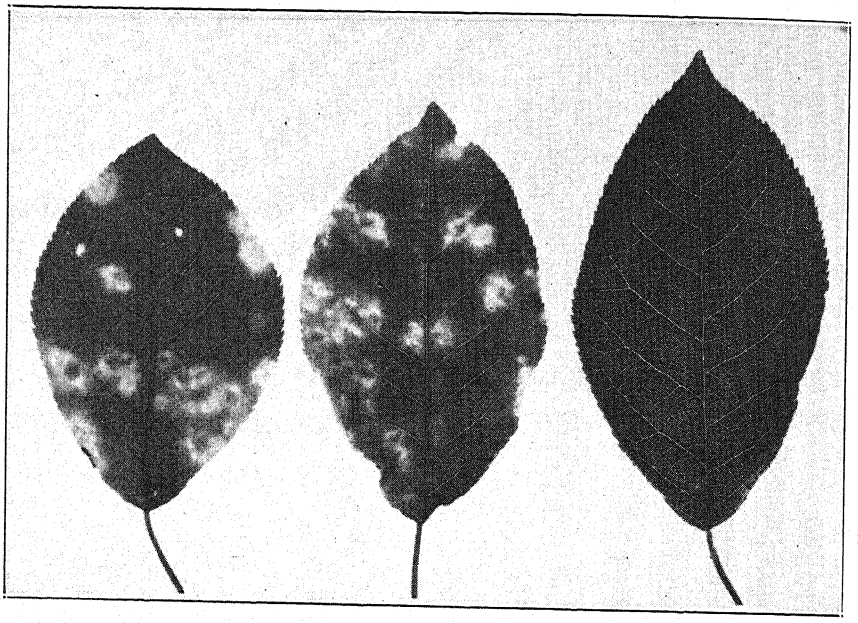


FIG. 7. Leaf symptoms on *P. virginiana* to which the disease had been transmitted by budding from sour cherry. A healthy leaf from a control tree is shown at the right.

Buds from the infected mahaleb sprouts were inserted in 2 healthy sour cherry trees in 1939. In 1940, the branches in which the buds were placed showed typical leaf symptoms of yellows, while the more remote branches showed no symptoms (Fig. 6). Several trees, left as unbudded controls, remained healthy.

*Prunus avium.* In 1939, buds from mazzard and 2 unknown cultivated varieties of sweet cherry were inserted in both healthy and diseased sour cherry trees. Six shoots grew from the buds placed in healthy trees and 7 from those placed in diseased trees. No definite symptoms of the disease appeared on any of these shoots in 1940.

*Prunus virginiana* L. In 1939, diseased sour cherry buds were inserted in several healthy chokecherry sprouts at one end of a large group. The re-

maining sprouts were not budded. Beginning in early June of 1940, leaves of all the budded sprouts showed abundant chlorotic ring spotting (Fig. 7). The affected leaves remained on the trees throughout the summer and did not develop the red color characteristic of chokecherry leaves affected by the X disease. The leaves of the control sprouts remained healthy. Similar ring spotting of natural occurrence on chokecherry (Fig. 8) was observed in 4 widely separated areas on the Door County Peninsula in 1940.

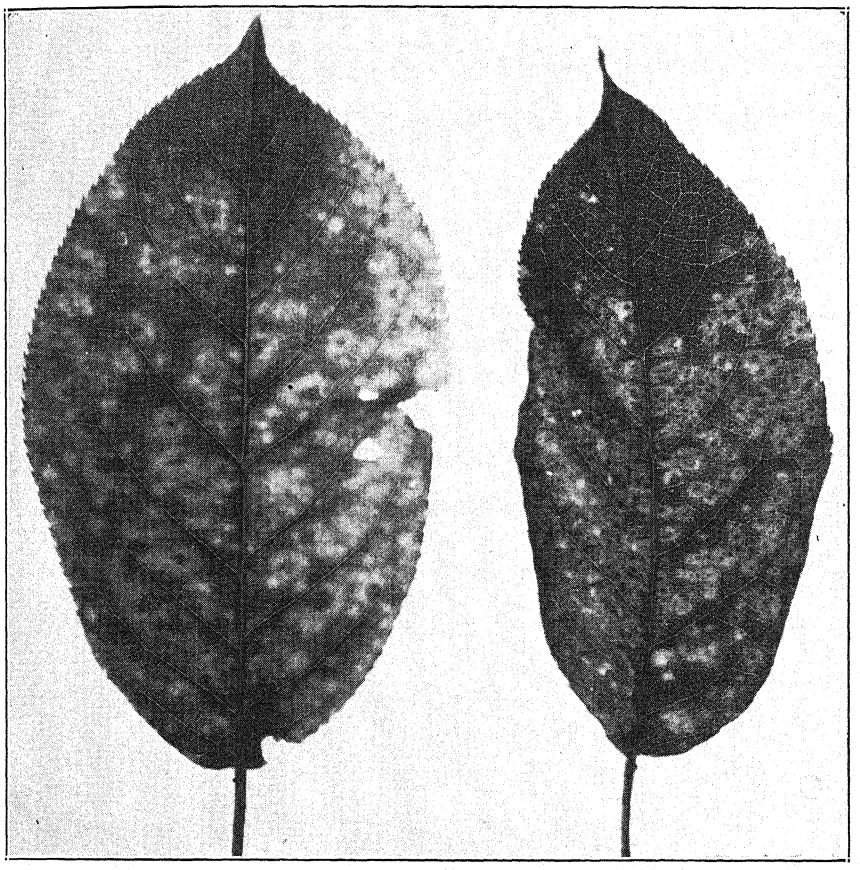


FIG. 8. Naturally occurring symptoms on leaves of a tree of *P. virginiana* located near a sour cherry orchard in which yellows was prevalent.

*Prunus pennsylvanica* L. Buds from diseased sour cherry trees were inserted in six healthy pin cherry sprouts in 1939. Union of tissues occurred on 2 of these. Chlorosis began to appear in the leaves of these 2 trees in June, 1940. Early in July some of the basal leaves dropped. The chlorotic areas on these leaves were at first light green, later yellow or pink. The leaves that remained on the trees showed light green chlorotic areas. The other 4 budded sprouts, and 6 similar unbudded ones, did not develop chlorosis. Similar chlorotic symptoms of natural occurrence were observed

on 1 pin cherry tree adjacent to a sour cherry orchard in which diseased trees were prevalent.

*Prunus serotina* Ehrh. Buds from diseased sour cherry were inserted in 2 healthy black cherry sprouts, and several similar sprouts were left unbudded. Union of tissues of bud-piece and sprout occurred in 1 case. In 1940, the basal leaves of this sprout showed light green chlorotic areas. These leaves were lighter green than normal and dropped in late June or early July. The unbudded sprouts showed no chlorosis. One black cherry tree showed naturally occurring symptoms similar to the chlorosis of the budded black cherry.

*Discussion.* The evidence that has been presented shows conclusively that the yellows disease is bud-transmissible from *P. cerasus* to *P. mahaleb* and back to *P. cerasus*. It is not yet certain whether the chlorotic symptoms incited on *P. mahaleb* are attributable to the yellows virus or to some associated virus or a virus complex. The data strongly suggest that the yellows virus or some associated virus or virus complex is bud-transmissible from sour cherry to *P. virginiana, P. pennsylvanica,* and *P. serotina.* The results with *P. avium* were negative, but subsequent experiments, which will be reported in a later paper, have shown that this species may carry the yellows virus.

In a recent paper Rasmussen and Cation (19) report transmitting cherry yellows from cherry to peach, peach to peach, and peach to cherry. They state that 3 strains of cherry yellows virus were differentiated by their reactions on peach and mahaleb cherry. Hildebrand (8)[4] reports that cherry yellows indexes readily on peach seedings, symptoms appearing in the greenhouse within 3 weeks after budding. Results similar to Hildebrand's have been obtained in greenhouse experiments now in progress with budded peach stock at Wisconsin. As has been noted by Hildebrand, Berkeley, and Cation (9), similar symptoms appear on peach trees affected by the severe ring spot virosis described by Cochran and Hutchins (5). Thomas and Rawlins (22) report a leaf mottle of sour cherry. Further work will be required to clarify the relationships of cherry yellows to these diseases and other viroses on stone fruits (*cf.* 9).

## SPREAD OF THE DISEASE IN THE ORCHARD

### Records of Incidence of the Disease

A record was taken annually from 1936 to 1940, inclusive, on each tree in 5 orchards in the vicinity of Sturgeon Bay, containing a total of 2,593 trees (Montmorency and Early Richmond, 11 to 29 years old in 1940). The observations were made during the major wave of defoliation. Each tree was classified as healthy, doubtfully or slightly diseased, moderately diseased, or severely diseased. Naturally, the classification of the stage of the disease varied somewhat with the time of observation and with the seasonal conditions.

[4] A fuller account of this work has appeared since the present paper was completed. (Hildebrand, E. M. Indexing cherry yellows on peach. Phytopath. 32: 712–719. 1942.)

TABLE 4.—*Rate of spread of cherry yellows in 5 orchards, Sturgeon Bay, Wis., 1936–1940*

| Orchard No. | Variety | Age of trees in 1940 | Trees observed | Trees moderately or severely diseased in stated years: | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 1936 | 1937 | 1938 | 1939[a] | 1940 |
| | | *Years* | *Number* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 1 | Montmorency | 16 | 1250 | 7 | 7 | 10 | 16 | 18 |
| 2 | Montmorency | 15 | 207 | 7 | 11 | 26 | 26 | 33 |
| 3 | Montmorency | 11 | 372 | 1 | 1 | 5 | 8 | 9 |
| 4 | Montmorency | 19 | 240 | 0 | 1 | 4 | 7 | 7 |
| 5 | Montmorency | 29 | 220 | 2 | 4 | 5 | 8 | 8 |
| 2 | Early Richmond | 15 | 134 | 7 | 9 | 12 | 18 | 36 |
| 5 | Early Richmond | 29 | 170 | 0 | 1 | 1 | 5 | 6 |
| | Total and weighted Avs. | ..... | 2593 | 4.6 | 5.8 | 7.4 | 13.2 | 16.6 |
| | Av. increase over preceding year | ..... | ..... | ..... | 1.2 | 1.6 | 5.8 | 3.4 |

[a] In 1939 and 1940, the "moderate" class included some trees that would have been classed as "slight" in previous years.

The data, which are summarized in table 4, definitely establish the fact that the disease is spreading in the orchards. In 1936, 4.6 per cent of the trees in the 5 orchards were moderately or severely diseased, and in 1940, 16.6 per cent of the trees were thus diseased. The rate of spread varies widely with different orchards. The average yearly increase in incidence of the moderately and the severely diseased trees in the 5 orchards on which the records were taken was 3 per cent of the total number of trees.

The spread of the disease in a representative portion of orchard 1 of table 4 in each year from 1936 to 1940 is shown in figure 9. The percentage of trees moderately or severely diseased increased from 8 in 1936 to 22 in 1940. The increase varied from year to year, the average annual increase being 3.5 per cent of the total number of trees.

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| – | – | – | – | – | 6 | – | – | 9 | – | – | 8 | – | – | 0 | – | – | – | – | 6 |
| 9 | 9 | – | – | 9 | – | – | – | – | – | 6 | – | – | 9 | – | – | – | 8 | – | 6 |
| – | 8 | – | – | 0 | – | – | – | 0 | – | – | – | – | – | – | – | | 6 | – | – |
| – | – | – | – | – | – | – | – | 0 | – | – | – | – | – | – | – | – | – | 8 | – |
| 6 | – | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | | – | – | 6 |
| 6 | 6 | – | – | – | – | – | – | 8 | – | – | 6 | – | – | – | 6 | – | – | – | 0 |
| – | – | – | | – | – | – | – | – | – | – | 6 | 0 | – | – | 6 | 0 | – | 7 | – |
| – | – | – | – | – | – | – | – | 6 | – | – | 0 | – | – | – | – | 9 | – | – | – |
| 0 | – | 0 | – | – | | – | – | – | – | – | 8 | – | – | – | – | – | 6 | – | 6 |
| 0 | – | 7 | | 6 | 6 | 6 | – | – | – | 8 | – | – | – | – | 9 | – | – | – | – |
| 6 | 8 | 0 | 8 | 8 | – | – | – | – | – | – | – | | – | – | – | – | – | 8 | – |
| – | – | – | – | – | 8 | – | – | – | 8 | – | – | – | – | – | – | 0 | – | | – |
| 8 | – | – | – | – | – | – | 6 | – | – | – | – | – | – | 6 | – | – | – | – | 6 |
| | – | – | – | 9 | – | – | – | – | 8 | – | – | – | | – | – | 8 | – | – | – |
| – | – | 7 | – | – | – | – | – | – | 6 | – | 0 | – | – | 6 | – | 0 | – | – | 6 |
| | – | – | – | – | – | – | – | 9 | – | – | – | – | – | – | – | – | – | – | – |

NUMBER OF TREES OBSERVED, 310

PERCENTAGE OF TREES DEFINITELY DISEASED:

1936, 8 1937, 9 1938, 14 1939, 17 1940, 22

FIG. 9. Incidence[5] of cherry yellows in a portion of orchard 1 (Table 4), Montmorency variety, Sturgeon Bay, Wis., 1936–1940.

In 1940, records of the incidence of diseased trees in 18 orchards containing 6,588 trees from 1 to 30 years old were taken in the Door County peninsular district in the manner described above. The data are summarized in table 5. The percentage of the moderately and the severely

[5] Explanation of symbols: – = healthy trees; 6 = trees on which definite symptoms of the disease were observed in 1936, when the survey was initiated; 7, 8, 9, 0 = trees on which definite symptoms were first observed in 1937, 1938, 1939, and 1940, respectively.

TABLE 5.—*Incidence of cherry yellows in 18 orchards, Door County, Wis., 1940*

| Orchard No. | Variety | Age of trees in 1940 | Trees observed | Trees in different stages of the disease in 1940 | | | |
|---|---|---|---|---|---|---|---|
| | | | | Slight | Moderate | Severe | Total of moderate and severe |
| | | *Years* | *Number* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 1 | Montmorency | 1 | 240 | 1.6 | 0.4 | 0.0 | 0.4 |
| 2 | Early Richmond | 2 | 82 | 0.0 | 22.0 | 2.4 | 24.4 |
| 3 | Montmorency | 3 | 40 | 12.5 | 30.0 | 7.5 | 37.5 |
| 4 | Montmorency | 1 to 8 | 680 | 0.9 | 0.6 | 1.5 | 2.1 |
| 5 | Montmorency | 8 | 520 | 2.1 | 1.0 | 0.6 | 1.6 |
| 6 | Montmorency | 8 | 260 | 16.2 | 17.3 | 7.0 | 24.3 |
| 7 | Montmorency | 8 | 333 | 3.6 | 3.3 | 0.6 | 3.9 |
| 8 | Montmorency and Early Richmond | 9 | 807 | 1.1 | 1.2 | 2.0 | 3.2 |
| 9 | Early Richmond | 10 | 122 | 0.8 | 1.6 | 7.4 | 9.0 |
| 10 | Montmorency | 11 | 360 | 2.8 | 5.8 | 1.4 | 7.2 |
| 11 | Montmorency and Early Richmond | 15 | 344 | 9.0 | 24.7 | 13.9 | 38.6 |
| 12 | Montmorency | 16 | 434 | 1.8 | 2.0 | 8.3 | 10.3 |
| 13 | Montmorency | 16 | 1250 | 3.7 | 9.7 | 7.4 | 17.1 |
| 14 | Montmorency and Early Richmond | 10 to 30 | 161 | 0.6 | 3.1 | 5.0 | 8.1 |
| 15 | Montmorency and Early Richmond | 20 | 354 | 1.1 | 1.1 | 7.6 | 8.7 |
| 16 | Montmorency and Early Richmond | 10 to 30 | 156 | 1.9 | 6.4 | 23.1 | 29.5 |
| 17 | Montmorency and Early Richmond | 10 to 30 | 125 | 0.6 | 0.6 | 5.6 | 6.2 |
| 18 | Montmorency and Early Richmond | 29 | 320 | 1.6 | 3.8 | 2.8 | 6.6 |
| | Total and weighted Avs. | ..... | 6588 | 3.0 | 5.7 | 5.0 | 10.7 |

diseased trees in the several orchards varied from 0.4 to 38.6, the weighted average for all orchards being 10.7 per cent. There is very little evidence of correlation between the age of the orchard and the percentage of diseased trees. However, there is probably a correlation between the number of trees diseased when received from the nursery and the number of diseased trees in the orchard. For instance, the nursery stock used for planting orchard 1 must have been nearly free from the disease, while that used for planting orchards 2 and 3 evidently included a considerable number of diseased trees. Furthermore, there is evidence that demand for large-fruited strains of sour cherry has led to some selection of buds from diseased trees. Orchards 6 and 7 are adjacent blocks, planted at the same time and in the same manner. The trees in orchard 6 were specially propagated as large-fruited strains of Montmorency. Those of orchard 7 were ordinary Montmorency. It is noteworthy that at the time of observation the percentage of diseased trees in the former orchard was 6 times as great as in the latter.

The rate of incidence of the disease appears to be about the same on Montmorency and Early Richmond; and trees of all ages seem susceptible (the disease was transmitted by budding to trees ranging from 1 to 29 years old).

These data show that the disease is steadily spreading in the orchards but indicate that the rate of spread is less rapid than is the case with some other virus diseases of stone fruits.

### Preliminary Experiments on Insect Transmission[6]

In August, 1939, a preliminary experiment was initiated to seek evidence on whether leaf hoppers (Cicadellidae) transmit the disease. Leaf hoppers of a mixed collection, predominantly of 3 species, taken from apple trees, were caged from 1 to several days on diseased Montmorency cherry trees, then transferred for 8- to 15-day periods to caged branches of 1-year-old Montmorency trees in the planting used in 1939 for experiments on transmission of the disease by budding. The same untreated trees that served as controls for the budding experiments were used as controls. In 1940, 4 of the 10 trees that had been subjected to infestation by leaf hoppers that had fed on diseased trees were definitely diseased, 4 showed doubtful results, and 2 showed no evidence of the symptoms. Of the 22 untreated controls, 16 were healthy, 6 were doubtful, and none were definitely diseased. As stated above, the occurrence of leaf spot on these trees complicated the readings. It is thought that the control trees recorded as doubtful are much more likely to have been free of the virus disease than to have had it.

Though confirmation and extension of this preliminary experimentation will be necessary before final conclusions are justified, the results give very strong evidence that the disease can be transmitted by leaf hoppers. Further experiments are in progress.

Preliminary experiments were initiated in 1939 to test the possibility that the black cherry aphid (*Myzus cerasi* Fab.) might transmit the disease. Aphids taken on July 3 from naturally infected sour cherry trees were placed on the leaves of 6 one-year-old Montmorency trees that were entirely enclosed in cheesecloth cages. Large colonies of aphids developed on leaves of 2 of these trees. Two weeks after the aphids had been placed on the leaves, the cages were removed and the trees sprayed with a nicotine sulphate solution to destroy the insects. No symptoms of the disease were observed in 1940 on these trees or on 10 similar untreated trees that served as controls.

In other experiments in 1939, black cherry aphids were transferred from diseased sour cherry trees to 23 healthy ones, most of which were 1 to 5 years old. There was little natural occurrence of the disease in the orchards in which the tests were made. Approximately 25 to 300 aphids were placed

[6] The work on insect transmission is being done in cooperation with John H. Lilly, Department of Economic Entomology, University of Wisconsin, and this preliminary statement is published with his approval.

on each tree, and no cages were used. In 1940, all of the 23 trees remained healthy, as did 10 untreated trees.

The results of these experiments, though not conclusive, give strong evidence that the disease is not transmitted by the black cherry aphid.

## CONTROL MEASURES

Recommendation of a complete program for control of cherry yellows must await the results of further studies. However, the available information clearly indicates certain steps that should be taken promptly. Since spread of the disease through nursery stock is an important source for its establishment in the orchard, the production and use of disease-free nursery stock is of prime importance. Young orchard plantings should be systematically inspected and diseased trees promptly removed when symptoms appear. In older orchards in which comparatively few trees are diseased, it would seem advisable similarly to remove the diseased trees in order to lessen the chances for spread of the trouble. Recommendations regarding older orchards in which the disease is well established are withheld until a fuller knowledge of the disease can be obtained. In the meantime, growers are advised to mark and observe all trees that show symptoms of yellows.

## SUMMARY

A destructive disease of sour cherry (*Prunus cerasus*), tentatively named cherry yellows, is incited by a virus or viruses. Affected trees tend to have relatively large leaves, some of which develop conspicuous chlorotic areas. The chlorotic leaves and some that are still green are abscised, a major wave of defoliation usually occurring about 3 or 4 weeks after petal-fall. Diseased trees probably live nearly as long as healthy ones but tend to have fewer spurs and sparse crops.

Reciprocal budding experiments between diseased and healthy sour cherry trees resulted in transmission of the disease in all cases in which union of tissues of bud-piece and budded tree was observed.

The virus was transmitted also by budding from *P. cerasus* to *P. mahaleb* and back to *P. cerasus,* though it is not certain whether the chlorotic symptoms incited on *P. hamaleb* are attributable to the yellows virus. Limited experiments on transmission to other *Prunus* species are reported.

Records of incidence of moderately or severely diseased trees, taken from 1936 to 1940, inclusive, in 5 orchards containing 2,593 trees, showed an average annual increase of 3 per cent. In 1940, the percentage of such diseased trees in 18 orchards, containing 6,588 trees, was 10.7. Montmorency and Early Richmond, the varieties dealt with in this work, appear to be equally susceptible to the disease.

Preliminary experiments gave strong though not conclusive evidence that the disease can be transmitted by leaf hoppers (Cicadellidae). Simi-

lar experiments with the black cherry aphid (*Myzus cerasi* Fab.) gave only negative results.

Further experiments on this disease and its control are in progress.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

LITERATURE CITED

1. ATANASOFF, D. Mosaic disease of drupaceous fruit trees. Sofia Univ., Faculty of Agr., Yearbook 13 (1, 1934–1935): 9–42. 1935.
2. CHRISTOFF, ALEXANDER. Virus diseases of the genus *Prunus* in Bulgaria. Phytopath. Ztschr. 11: 360–422. 1938.
3. CLARK, NOBLE, and NIEMEN HOVELAND. A destructive disease menaces sour cherries. *In* What's new in farm science (Ann. Rept. of Dir. 56, Pt. II, 1938–39). Wis. Agr. Exp. Stat. Bull. 449: 25–26. 1940.
4. ————, and ————. Yellows of sour cherry a virus disease. *In* What's new in farm science (Ann. Rept. of Dir. 58, Pt. II, 1940–41). Wis. Agr. Exp. Stat. Bull. 455: 71–72. 1942.
5. COCHRAN, L. C., and LEE M. HUTCHINS. A severe ring-spot virosis on peach. (Abstract) Phytopath. 31: 860. 1941.
6. GLOYER, W. O., and HUGH GLASGOW. Defoliation of cherry trees in relation to winter injury. N. Y. (Geneva) Agr. Exp. Stat. Bull. 555, 27 pp. 1928.
7. HEALD, F. D., L. K. JONES, and G. A. HUBER. Plant disease survey. Wash. Agr. Exp. Stat. Ann. Rept. 44: 50–51. 1934.
8. HILDEBRAND, E. M. Rapid transmission techniques for stone-fruit viruses. Science (n.s.) 95: 52. 1942.
9. ————, G. H. BERKELEY, and D. CATION. Handbook of virus diseases of stone fruits in North America. Mich. Agr. Exp. Stat. Misc. Pub., 76 pp. 1942.
10. ————, and W. D. MILLS. Cherry yellows (physiological yellow leaf) in New York. Phytopath. 31: 355–356. 1941.
11. KEITT, G. W., E. C. BLODGETT, E. E. WILSON, and R. O. MAGIE. The epidemiology and control of cherry leaf spot. Wis. Agr. Exp. Stat. Res. Bull. 132, 117 pp. 1937.
12. ————, and C. N. CLAYTON. A destructive bud-transmissible disease of sour cherry in Wisconsin. Phytopath. 29: 821–822. 1939.
13. ————, and ————. A bud-transmissible chlorosis of *Prunus cerasus*. (Abstract) Phytopath. 30: 13. 1940.
14. ————, and ————. A destructive bud-transmissible disease of sour cherry. Wis. Hort. 30: 276. 1940.
15. ————, and ————. Further experiments on a virus disease of *Prunus cerasus*. (Abstract) Phytopath. 31: 14. 1941.
16. MAERZ, A., and M. REA PAUL. A dictionary of color. McGraw-Hill Book Company, Inc. (New York). 207 pp. 1930.
17. RASMUSSEN, E. J. Causes of premature defoliation of sour cherry. Oreg. State Hort. Soc. Ann. Rept. 30: 131–137. 1938.
18. ————, and DONALD CATION. A progress report on the yellow-leaf disease of sour cherry. Mich. State Hort. Soc. Ann. Rept. 71: 100–105. 1942.
19. ————, and ————. Experiments on the yellows disease of sour cherry (*Prunus cerasus*). (Abstract) Phytopath. 32: 15. 1942.
20. REEVES, E. L., GLENN A. HUBER, and KARL E. BAUR. "Pink fruit" or necrosis of sour cherry in western Washington. U. S. Dept. Agr. Plant Dis. Rptr. 23: 10–12. 1939.
21. STEWART, F. C. Notes on New York plant diseases, II. N. Y. (Geneva) Agr. Exp. Stat. Bull. 463: 157–188. 1919.
22. THOMAS, H. EARL, and T. E. RAWLINS. Some mosaic diseases of *Prunus* species. Hilgardia 12: 623–644. 1939.

# PATHOGENICITY STUDIES WITH FUSARIA ISOLATED FROM TOBACCO, SWEET POTATO AND COTTON[1]

T. E. SMITH AND K. J. SHAW[2]

(Accepted for publication August 18, 1942)

## INTRODUCTION

Fusarium wilt of tobacco (*Nicotiana tabacum* L.), commonly attributed to *Fusarium oxysporum* Schl. v. *nicotianae* Johnson (10), is a minor disease of flue-cured tobacco. It is of economic importance at present only in Columbus County, North Carolina, near Whiteville and Chadbourn, where it has been present since about 1931. The disease is becoming more serious as evidenced by spread during recent years in this and other areas. Fusarium wilt of tobacco also has been reported from Maryland, Ohio, Kentucky, Canada, South Africa, and Russia. The disease was attributed to *F. oxysporum nicotianae* in all reports, but, in South Africa, Doidge (7) also isolated in one instance, *F. bulbigenum* from all parts of the discolored wood from roots to petiole. Fusarium wilt of cotton (*Gossypium hirsutum* L.), commonly attributed to *F. vasinfectum* Atk., is a major disease, more or less universally present wherever this crop is grown. Fusarium wilt or stem rot of sweet potato (*Ipomea batatas* (L.) Lam.), commonly attributed to *F. bulbigenum* Cke. and Mass. v. *batatas* Wr. and *F. oxysporum* Schl. f. 2 Wr., is a major disease of this crop and is also more or less universally present. In the flue-cured tobacco belt, cotton, sweet potato, and tobacco are often grown in rotations on the same fields. The host range of Fusaria from these crops was originally considered different, hence their use in rotations was unlimited. However, the work of Armstrong (1, 2, 3) and others raised several questions about the indiscriminate growth of these crops on the same soil during successive years.

## RELATION OF SWEET POTATO ROTATIONS TO OCCURRENCE OF FUSARIUM WILT IN FLUE-CURED TOBACCO

At Creedmoor, North Carolina, a few tobacco plants developed fusarium wilt when grown in a rotation experiment with Granville wilt (*Bacterium solanacearum* E.F.S.). The fusarium-infected plants were found in 1940 on a plot where sweet potato was grown in 1936 and 1937, tobacco in 1938 and sweet potato in 1939. No fusarium wilt developed on tobacco grown after redtop grass, crab grass, corn, native weeds, or tobacco. Isolations of *Fusarium* from tobacco were pathogenic to tobacco and sweet potato. This was the only known occurrence of fusarium wilt in the Creedmoor area.

[1] Cooperative Investigation by U. S. Department of Agriculture, N. C. Agricultural Experiment Station and the N. C. Department of Agriculture. Published with the approval of the Director, North Carolina Agricultural Experiment Station, as paper no. 146 of the Journal Series.

[2] The writers express appreciation for the cooperation of many persons who sent collections of plant material and cultures or allowed use of their unpublished observations.

At the McCullers Branch Station, McCullers, North Carolina, fusarium wilt of tobacco first appeared in 1938 on a plot planted in 1937 to sweet potato heavily inoculated with a sweet-potato isolate collected in New Jersey in 1925 by R. F. Poole.[3] A part of this plot was planted to tobacco in 1939, 1940, and 1941, and fusarium wilt was present every year, reaching a maximum of 50 per cent kill. Cotton, soybeans, and sweet potato have have also been grown on this plot in rotation with tobacco but only tobacco and sweet potato had fusarium wilt. In 1940, fusarium wilt of tobacco appeared in a crop-rotation experiment being conducted on the root-knot disease in a different area of the farm. This experiment was a randomized block design with 10 rotations in 4 blocks and the fusarium wilt was present on tobacco in all 4 plots previously planted to sweet potato, but was absent on tobacco grown after corn, cotton, peanuts, velvet beans, soybeans, crotalaria, native weeds, oats and native weeds, and bare fallow. The plots were enclosed with creosoted-pine boards to control surface water and all cultivation was done with well-cleaned hand tools. Therefore, it was unlikely that the disease was introduced by surface water or contaminated implements. A small amount of fusarium wilt had been observed on sweet potato whenever this crop was grown.

A few tobacco plants with fusarium wilt were observed in 1940 on a field planted in 1939 to sweet potato at the Pee Dee Experiment Station, Florence, South Carolina (reported by T. W. Graham[3]).

Similar observations were made at the Georgia Coastal Plain Experiment Station, Tifton, Georgia, by J. G. Gaines.[3] In a rotation experiment being conducted on the root knot disease, fusarium wilt of tobacco was observed on a plot where sweet potato was grown the previous year. No fusarium wilt developed on tobacco grown after cowpeas, cotton, corn, peanuts, velvet beans or native weeds on other plots of the experiment. The cotton was badly wilted during some years. In 1930, 1931, 1932, and 1933, cotton was killed 34 to 55 per cent but tobacco grown the succeeding years showed no wilt. Thus, fusarium wilt of flue-cured tobacco was associated with sweet potato rotations at 4 locations in 3 States. But no wilt was observed on tobacco grown after cotton at these locations.

### OBSERVATIONS ON THE OCCURRENCE OF FUSARIUM WILT OF BURLEY TOBACCO

Fusarium wilt of Burley tobacco was reported by Armstrong (1) at 6 locations where there was no record of previous culture of this crop. The tobacco was grown on cotton farms and it was later demonstrated that Fusaria from cotton, okra, and coffee weed (*Cassia tora*) were pathogenic to Burley tobacco. Similar observations were made by E. E. Clayton,[3] who, in 1941, found fusarium-infected Burley in Northeastern Virginia, where, likewise, there was no record of previous tobacco culture. In addition to these observations, fusarium wilt has been generally found on Burley in plantings made for experimental purposes throughout the Southeastern

[3] Personal communications.

States. It appears that fusarium wilt of Burley was associated with rotations of cotton and perhaps other plants.

## PATHOGENICITY TRIALS WITH FUSARIA ISOLATED FROM TOBACCO, SWEET POTATO AND COTTON

### Methods

Several different methods of inoculation were tried: (1) Stem inoculation with bits of discolored woody tissue from diseased plants. (2) Soil inoculation with cultures grown on cornmeal-sand medium. (3) Soil inoculation with macerated diseased stems. (4) Root inoculation made by pouring diluted cultures in holes punched in the soil around potted plants. (5) Dipping the roots of plants, prior to transplanting, in a diluted culture. Some infection was obtained with methods 1, 2, 3, and 4, but the results were not consistent. Method 5, a modification of that described by Wellman (16), was finally adopted because of its simplicity and the fact that the results could be duplicated in repeated trials.

Fusaria were isolated by the writers from fresh specimens of wilted plants, designated by collection numbers in the tables, or cultures were obtained from other workers as indicated. Inoculum was prepared by diluting macerated agar-slant cultures or by diluting liquid cultures grown on the mineral-nutrient solution plus 1 per cent dextrose. Preliminary trials showed that dilutions of 1 part culture to 5 or 20 parts total volume made no difference in the end effect. The inoculation technique was to rinse the loosely-held soil from the roots, dip them in diluted inoculum and set the plants into steam-sterilized sand in pots or flats. Unwashed sand obtained from streams and roadside ditches was used. Inoculated plants were carefully and adequately protected from accidental contamination by other Fusaria, as illustrated by the fact that the noninoculated controls, approximately 40 in all, remained healthy. The plants were watered once daily with nutrient solution and with tap water as often as required. The nutrient solution was prepared by adding boron, manganese, and iron, in proper amount, to the stock solution used by McMurtrey (11).

Fusarium-susceptible strains of test plants were used for all inoculations. The Jamaica variety of flue-cured tobacco, Judy Pride variety of Burley tobacco, Porto Rico variety of sweet potato, and Half and Half or Cokers 100–1 varieties of cotton were grown. These agronomic varieties were employed because they are in general use by farmers in the Southeastern States and are representative of the fusarium-susceptible strains of their respective crops. Seedlings of tobacco and cotton were grown from surface-sterilized pure-bred seed. Cotton was transplanted with ease if taken up before the lateral roots became more than 1 or 2 inches long. Sprouts of sweet-potato roots or rooted cuttings of field-grown vines were used for inoculations on this plant.

In recording the results of inoculations, reliance was placed in the type of symptoms observed on the various plants following natural infection

under field conditions. In most cases this was possible because the plants made normal growth before wilt symptoms developed. Thus, pathogenicity on tobacco and sweet potato was characterized at first by yellowing of the leaves, followed later by wilting and in severe cases death. On cotton the first symptom was wilting of the green leaves. Extensive discoloration of the stele occurred almost simultaneously with wilting. The results on Burley tobacco, sweet potato, and cotton were clear-cut, that is, these symptoms were either present or absent and are shown in the tables as "+" and "−", respectively. On flue-cured tobacco, however, an intermediate reaction often was obtained, which was characterized by wilting of leaves while still green or by severe stunting. These symptoms have not been associated with fusarium wilt of tobacco under field conditions and were not considered typical pathogenic effects, but atypical symptoms resulting from heavy inoculations. Therefore, the intermediate reaction, indicated in the tables by "±," was largely ignored in interpreting the results. Some pathogenic effects, such as limited discoloration of the stele and slight stunting, followed nearly all inoculations classed as negative, but these were disregarded in recording the results.

Inoculation trials were conducted from August, 1940, through January, 1942, in the greenhouse at the Tobacco Branch Station, Oxford, North Carolina. Air temperatures varied from a maximum of 110° F. on bright days in mid-summer to a minimum of about 60° F. at night and on cloudy days in mid-winter. Results obtained under the higher temperatures of summer were duplicated many times under the lower temperatures of mid-winter, by allowing a longer period of time to elapse between inoculation and final note-taking. In summer a 15-day period was often adequate, but in winter 30 or 45 days were often required for the development of decisive results. The accuracy of the observations was repeatedly verified by growing the plants to maturity.

### Inoculations with Fusaria from Tobacco

Fifty-three collections from various commercial types of tobacco from 5 States were tested and are considered representative of fusarium wilt of tobacco as it occurs in this country. The results of inoculations on flue-cured tobacco, Burley tobacco, sweet potato, and cotton are summarized in table 1. The collections are tabulated by type of tobacco and by State of origin for convenience in reviewing the results. Two different physiologic races were collected: (1) The race pathogenic on flue-cured tobacco, Burley tobacco, and sweet potato but nonpathogenic on cotton. (2) The race pathogenic on Burley tobacco and cotton and giving the intermediate reaction on flue-cured tobacco, in most cases, but nonpathogenic on sweet potato. In repeated trials all isolations were remarkably consistent except on flue-cured tobacco. There was a tendency for certain collections, during storage or following reisolation, to lose virulence for this test plant, even though they were originally collected, in some cases, from this type of tobacco.

TABLE 1.—*Results of inoculations with Fusaria isolated from tobacco*

| Collection and year originally isolated | Type of tobacco | Collected at | Result of inoculations on | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Flue-cured tobacco | | | Burley tobacco | | Sweet potato | | Cotton |
| | | | +[a] | ± | − | + | − | + | − | + − |
| 1; 1940 | Flue-cured | Creedmoor, N. C. | 5[b] | 0 | 0 | 1 | 0 | 5 | 0 | 0 4 |
| 7; 1940 | do. | Chadbourn, N. C. | 17 | 0 | 0 | 2 | 0 | 17 | 0 | 0 16 |
| 8; 1940 | do. | Whiteville, N. C. | 2 | 0 | 0 | 1 | 0 | 2 | 0 | 0 1 |
| 9; 1940 | do. | do. | 3 | 0 | 0 | 1 | 0 | 3 | 0 | 0 3 |
| 10; 1940 | do. | do. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 1 |
| 11; 1940 | do. | do. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 2 |
| 12; 1940 | do. | do. | 3 | 0 | 0 | 1 | 0 | 3 | 0 | 0 3 |
| 13; 1940 | do. | do. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 2 |
| 14; 1940 | do. | do. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 2 |
| 15; 1940 | do. | do. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 2 |
| 19; 1940 | do. | McCullers, N. C. | 1 | 0 | 2 | 1 | 0 | 3 | 0 | 0 2 |
| 20; 1940 | do. | do. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 2 |
| 69; 1941 | do. | Chadbourn, N. C. | 1 | 0 | 0 | − | − | 1 | 0 | 0 1 |
| 92; 1941 | do. | Whiteville, N. C. | 2 | 0 | 0 | 1 | 0 | 2 | 0 | 0 3 |
| 99; 1941 | do. | do. | 2 | 0 | 0 | 1 | 0 | 2 | 0 | 0 2 |
| 2; 1940 | do. | Florence, S. C. | 3 | 0 | 0 | 1 | 0 | 3 | 0 | 0 3 |
| 4; 1940 | do. | Tifton, Ga. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 2 |
| 6; 1940 | do. | do. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 2 |
| 103; 1941 | do. | do. | 2 | 0 | 0 | 1 | 0 | 2 | 0 | 0 3 |
| 104; 1941 | do. | do. | 2 | 0 | 0 | 1 | 0 | 2 | 0 | 0 2 |
| 105; 1941 | do. | do. | 0 | 4 | 0 | 3 | 0 | 0 | 4 | 4 0 |
| 33; 1940 | Maryland | Upper Marlboro, Md. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 2 |
| 34; 1940 | do. | Maryland | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 2 |
| 35; 1940 | do. | do. | 3 | 0 | 0 | 1 | 0 | 3 | 0 | 0 3 |
| 36; 1940 | do. | do. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 2 |
| 107; 1941 | do. | do. | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 2 |
| E. A. Walker's single spore cultures; 1939 | do. | Charles Co., Md. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 3 |
| | do. | do. | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 1 |
| | do. | do. | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 1 |
| | do. | do. | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 1 |
| 130; 1941 | do. | do. | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 1 |
| E. M. Johnson's culture; 1937 | do. | Florence. S. C. | 0 | 1 | 1 | 2 | 0 | 0 | 2 | 2 0 |
| G. M. Armstrong's culture | Burley | South Carolina | 0 | 3 | 3 | 3 | 0 | 0 | 6 | 6 0 |
| 108; 1941 | do. | do. | 0 | 2 | 1 | 1 | 0 | 0 | 3 | 3 0 |
| E. M. Johnson's culture; 1937 | do. | Lexington Ky. | 1 | 0 | 0 | − | − | 1 | 0 | 0 2 |
| Do.; 1937 | do. | do. | 2 | 0 | 0 | 1 | 0 | 2 | 0 | 0 3 |
| Do.; 1937 | do. | do. | 2 | 0 | 0 | 1 | 0 | 2 | 0 | 0 2 |
| Do.; 1937 | do. | do. | 2 | 0 | 0 | 1 | 0 | 2 | 0 | 0 2 |
| Do.; 1937 | do. | do. | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 1 |
| | do. | do. | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 0 |

TABLE 1.—*Concluded*

| Collection and year originally isolated | Type of tobacco | Collected at | Result of inoculations on | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Flue-cured tobacco | | | Burley tobacco | | Sweet potato | | Cotton | |
| | | | +[a] | ± | − | + | − | + | − | + | − |
| E. M. Johnson's culture; 1935 | Burley | Carter City, Ky. | 2 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 2 |
| Do.; 1935 | do. | Middlesboro, Ky. | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 3 | 0 |
| Do.; 1935 | do. | Spurlington, Ky. | 2 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 3 |
| Do.; 1935 | do. | Maysville, Ky. | 3 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 3 |
| Do.; 1937 | do. | Logan Co., Ky. | 0 | 2 | 0 | 1 | 0 | 0 | 2 | 2 | 0 |
| Do.; 1937 | do. | Todd Co., Ky. | 0 | 2 | 0 | 1 | 0 | 0 | 2 | 3 | 0 |
| Do.; 1937 | do. | do. | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 |
| Do.; 1937 | do. | Jefferson Co., Ky. | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| Do.; 1937 | do. | Christian Co., Ky. | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| Do.; 1933 | do. | Mercer Co., Ky. | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| Do.; 1935 | do. | Boone Co., Ky. | 1 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 2 |
| Do.; 1937 | One-sucker dark | Logan Co., Ky. | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 2 | 0 |
| Do.; 1937 | do. | Todd Co., Ky. | 0 | 1 | 1 | 2 | 0 | 0 | 2 | 2 | 0 |
| Do.; 1937 | do. | Simpson Co., Ky. | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 |
| Do.; 1931 | Dark-fire-cured | Graves Co., Ky. | 1 | 1 | 1 | 3 | 0 | 3 | 0 | 0 | 4 |
| Do.; 1937 | do. | Simpson Co., Ky. | 0 | 0 | 1 | 0 | 2 | 0 | 1 | 2 | 0 |
| Do.; 1937 | do. | Logan Co., Ky. | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 |

[a] Explanation of methods used in recording results: + means pathogenic, characterized by extensive discoloration of stele in combination with yellows and wilt on tobacco and sweet potato or wilt only on cotton. ± means intermediate reaction, characterized by severe stunting or slight wilt of green leaves. − means nonpathogenic.

[b] To obtain the total trials on each test plant, add the entries in each cell of the table.

Collection number 7 from Chadbourn, North Carolina, was the most virulent and this culture maintained virulence for flue-cured tobacco and sweet potato, without transfer, for 12 months.

In considering isolations from flue-cured tobacco, all the isolates except one (No. 105 from Tifton, Georgia) belong to the race pathogenic on sweet potatoes. Among isolates from Maryland tobacco, the race pathogenic on sweet potato also predominated, but one collection (Johnson's Culture; 1937) from the experimental plots at Florence, South Carolina, was pathogenic on cotton. From Burley, one-sucker, and dark-fire-cured tobaccos, the race pathogenic for tobacco and sweet potato and the race pathogenic for

Burley and cotton were obtained in approximately equal numbers. Armstrong's collection from Burley in South Carolina was pathogenic on cotton, as pointed out by that investigator (3).

## Results of Inoculations with Fusaria from Sweet Potato

Twenty-two collections from sweet potato were tested, 19 from widely scattered localities in North Carolina, 2 from South Carolina and 1 from Georgia. The results of inoculation trials are given in table 2. All

TABLE 2.—*Results of inoculations with Fusaria isolated from sweet potato*

| Collection and year originally isolated | Collected at | Results of inoculations on | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Flue-cured tobacco | | | Burley tobacco | | Sweet potato | | Cotton | |
| | | +[a] | ± | − | + | − | + | − | + | − |
| 41; 1940 | McCullers, N. C. | 1[b] | 0 | 1 | ..... | ..... | 2 | 0 | 0 | 1 |
| 42; 1940 | do. | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 1 |
| 43; 1940 | Creedmoor, N. C. | 0 | 1 | 1 | 1 | 0 | 2 | 0 | 0 | 1 |
| 51; 1940 | do. | 0 | 0 | 2 | 1 | 0 | 2 | 0 | 0 | 1 |
| 57; 1940 | Oxford, N. C. | 0 | 0 | 2 | 1 | 0 | 2 | 0 | 0 | 1 |
| 58; 1940 | Creedmoor, N. C. | 0 | 0 | 2 | 1 | 0 | 2 | 0 | 0 | 1 |
| 59; 1940 | do. | 0 | 0 | 3 | 1 | 0 | 3 | 0 | 0 | 2 |
| 60; 1940 | Raleigh, N. C. | 0 | 0 | 4 | 1 | 0 | 4 | 0 | 0 | 3 |
| 61; 1940 | do. | 1 | 5 | 7 | 1 | 0 | 13 | 0 | 0 | 13 |
| 62; 1940 | do. | 0 | 1 | 1 | 1 | 0 | 2 | 0 | 0 | 1 |
| 64; 1940 | Oxford, N. C. | 0 | 0 | 2 | 1 | 0 | 2 | 0 | 0 | 1 |
| 70; 1941 | Whiteville, N. C. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 2 |
| 80; 1941 | do. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 2 |
| 83; 1941 | Kinston, N. C. | 0 | 1 | 1 | 1 | 0 | 2 | 0 | 0 | 2 |
| 89; 1941 | do. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 2 |
| 91; 1941 | Dover, N. C. | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 2 |
| 93; 1941 | Whiteville, N. C. | 2 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 2 |
| 94; 1941 | Dover, N. C. | 0 | 0 | 2 | 1 | 0 | 2 | 0 | 0 | 2 |
| 95; 1941 | Clinton, N. C. | 1 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 2 |
| 72; 1941 | Florence, S. C. | 0 | 1 | 1 | 1 | 0 | 2 | 0 | 0 | 2 |
| 73; 1941 | Nichols, S. C. | 0 | 2 | 0 | 1 | 0 | 2 | 0 | 0 | 2 |
| 102; 1941 | Tifton, Ga. | 0 | 0 | 2 | 1 | 0 | 2 | 0 | 0 | 2 |

[a] Explanation of methods used in recording results: + means pathogenic, characterized by extensive discoloration of stele in combination with yellows and wilt on tobacco and sweet potato or wilt only on cotton. ± means intermediate reaction, characterized by severe stunting or slight wilt of green leaves. − means nonpathogenic.

[b] To obtain the total trials on each test plant, add the entries in each cell of the table.

collections were consistently pathogenic on Burley tobacco and sweet potato but nonpathogenic on cotton. On flue-cured tobacco, 8 of the 22 collections were able to cause typical fusarium wilt symptoms in one or more trials. Collections from McCullers and Whiteville, North Carolina, were expected to be pathogenic for tobacco because fusarium wilt on flue-cured tobacco is generally present in the Whiteville area and to a limited extent at McCullers. However, collections from areas far removed from known infestations of fusarium wilt of tobacco were pathogenic for flue-cured tobacco. For example, collections 89, 91, and 95 were made at locations approximately 30 to 90 miles from the nearest known tobacco infestations. These results explain the previously cited field observations by showing that

races pathogenic for flue-cured tobacco do occasionally occur in sweet potato. When, however, they are kept in culture, they generally lose virulence for flue-cured tobacco. The susceptibility of Burley tobacco to all sweet-potato collections was striking (Fig. 1). Complete yellowing of the

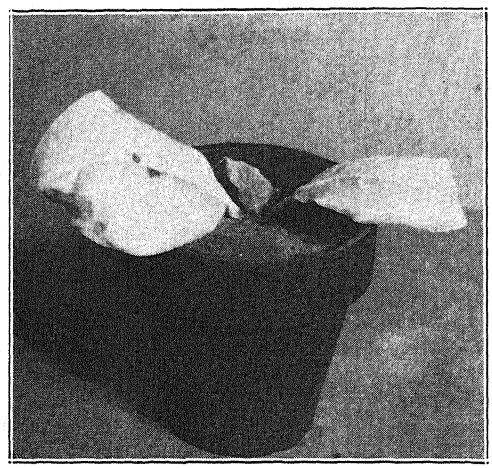


FIG. 1. Susceptibility of Burley tobacco to an isolate from sweet potato. General yellowing of the leaves occurred 5 to 10 days after inoculation and death followed within a few days. Similar results were obtained with all isolates from tobacco, sweet potato, and cotton.

leaves occurred 5 to 10 days after inoculation, and death followed immediately. In several different comparisons, there was a close parallel in the development of symptoms following inoculations on Burley with isolates from sweet potato, nonpathogenic for flue-cured tobacco, and isolates pathogenic for flue-cured tobacco. Burley tobacco, therefore, was as susceptible to races parasitic on sweet potato alone as to races parasitic to sweet potato and flue-cured tobacco.

### Results of Inoculation with Fusaria from Cotton

Nineteen collections from cotton were tested, of which 13 came from North Carolina, 5 from South Carolina, and 1 from Georgia. The results of inoculation trials are summarized in table 3. All collections were consistently pathogenic for Burley tobacco and cotton but nonpathogenic for sweet potato. Nearly all collections gave the intermediate reaction on flue-cured tobacco. However, in repeated trials with several isolates which appeared to have the most virulence for this test plant, no typical yellowing of the leaves was obtained. The results were in agreement with observations in the field, previously cited, and show that Burley was susceptible to the cotton *Fusarium*. Flue-cured tobacco was not susceptible to any cotton collection, but from the results with collection 105 (Table 1) it appears that the cotton race may parasitize this type of tobacco to a limited extent. The

TABLE 3.—*Results of inoculations with Fusaria isolated from cotton*

| Collection and year originally isolated | Collected at | Results of inoculations on | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Flue-cured tobacco | | | Burley tobacco | | Sweet potato | | Cotton | |
| | | +[a] | ± | − | + | − | + | − | + | − |
| 71; 1941 | Chadbourn, N. C. | 0[b] | 2 | 1 | 1 | 0 | 0 | 3 | 3 | 0 |
| 77; 1941 | Lumberton, N. C. | 0 | 2 | 1 | 1 | 0 | 0 | 3 | 3 | 0 |
| 78; 1941 | Whiteville, N. C. | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 1 | 1 |
| 79; 1941 | Fayetteville, N. C. | 0 | 3 | 0 | 1 | 0 | 0 | 3 | 3 | 0 |
| 82; 1941 | Chadbourn, N. C. | 0 | 2 | 1 | 1 | 0 | 0 | 3 | 2 | 1 |
| 84; 1941 | Kinston, N. C. | 0 | 3 | 1 | 2 | 0 | 0 | 4 | 4 | 0 |
| 87; 1941 | do. | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 2 | 0 |
| 88; 1941 | do. | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 2 | 0 |
| 96; 1941 | Elizabethtown, N. C. | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 2 | 0 |
| 97; 1941 | Kinston, N. C. | 0 | 2 | 1 | 1 | 0 | 0 | 2 | 3 | 0 |
| 98; 1941 | Elizabethtown, N. C. | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 2 | 0 |
| 100; 1941 | Mount Olive, N. C. | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 2 | 0 |
| 101; 1941 | Kinston, N. C. | 0 | 0 | 2 | 1 | 0 | 0 | 2 | 1 | 1 |
| 65; 1940 | Florence, S. C. | 0 | 0 | 2 | 1 | 0 | 0 | 2 | 2 | 0 |
| 66; 1940 | do. | 0 | 13 | 4 | 1 | 0 | 0 | 17 | 16 | 1 |
| 67; 1940 | do. | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 2 | 0 |
| 74; 1941 | Mullins, S. C. | 0 | 2 | 1 | 2 | 0 | 0 | 3 | 3 | 0 |
| Armstrong's cultures | South Carolina | 0 | 3 | 0 | 1 | 0 | 0 | 3 | 3 | 0 |
| | do. | 0 | 3 | 0 | 1 | 0 | 0 | 3 | 3 | 0 |
| 106; 1941 | Tifton, Ga. | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 2 | 0 |

[a] Explanation of methods used in recording results: + means pathogenic, characterized by extensive discoloration of stele in combination with yellows and wilt on tobacco and sweet potato or wilt only on cotton. ± means intermediate reaction, characterized by severe stunting or slight wilt of green leaves. − means nonpathogenic.

[b] To obtain the total trials on each test plant, add the entries in each cell of the table.

host range of all cotton collections was the same as that of the cotton-virulent race isolated many times from Burley and dark tobaccos but rarely from flue-cured and Maryland types.

### EXPERIMENTS ON THE STABILITY OF PATHOGENICITY DURING REPEATED INOCULATION AND REISOLATION FROM RESISTANT PLANTS AND DURING STORAGE

It was noted in many cases that invasion occurred after inoculation, but typical wilt symptoms did not develop. The question arose as to whether the pathogenicity of such weak invaders could be increased by repeated inoculation and reisolation. The question was first investigated with a collection originally isolated from flue-cured tobacco (No. 7, Table 1). This culture was inoculated into and reisolated from cotton 4 times. Attempts to reisolate the culture following the fourth inoculation were unsuccessful, and this series was discontinued with the conclusion that it had not increased in virulence for cotton but was still pathogenic for tobacco and sweet potato. A sweet-potato collection (No. 61, Table 2) gave the intermediate reaction on flue-cured tobacco when originally tested. During 7 passages through this type of tobacco, it varied in virulence from typically pathogenic, with the production of leaf yellowing, to apparently nonpathogenic. The typical symptoms developed following inoculation with the fifth reisolation in July,

1941, during an exceptionally hot period, when maximum temperatures averaged about 100° F. Reisolations gave the usual intermediate or negative reactions in later trials and it was concluded that virulence had not been increased. The culture retained its virulence for sweet potato throughout the 7 passages through tobacco. A cotton collection (No. 66, Table 3) also gave the intermediate reaction on flue-cured tobacco when originally tested. During 7 passages through tobacco, it varied from the intermediate reaction to apparently nonpathogenic but maintained virulence for cotton. From these tests, it was concluded that increases in virulence for resistant plants were not obtained by repeated inoculation and reisolation from them and that pathogenicity was a relatively stable characteristic under the experimental conditions provided. However, there was a partial loss of virulence in many collections during storage. Several isolates from tobacco or sweet potato that were pathogenic on flue-cured tobacco in the first test, were weakly or nonpathogenic in later trials on this test plant, but retained virulence for sweet potato throughout the storage period. An unusually interesting illustration of partial loss in virulence was one of Johnson's cultures isolated in 1937 from dark-fire-cured tobacco in Simpson County, Kentucky. This isolate, when tested in 1941, was nonpathogenic on either type of tobacco, but had retained virulence for cotton.

### MISCELLANEOUS INOCULATION TRIALS

Additional pathogenicity trials not reported in earlier sections of the paper were conducted, using the methods previously described. Flue-cured tobacco, sweet potato, and cotton were not susceptible to one isolate from wilted cowpeas collected in a field where fusarium wilt was present on tobacco. Tomato was not susceptible to one collection of the sweet-potato-virulent race from flue-cured tobacco. Tests with Irish potato were not conclusive because of the difficulty of obtaining fusarium-free plants. In two different sets of inoculations, the control plants developed symptoms characteristic of *Fusarium oxysporum* and *F. solani eumartii*, as described by Goss (8). It was clear, however, that Irish potato was not susceptible to all Fusaria from tobacco, because many plants remained healthy after inoculations with several tobacco collections. Cultures of 2 Fusaria from Irish potato were obtained from R. W. Goss of the Nebraska Agricultural Experiment Station. *F. oxysporum* Schl. was nonpathogenic in all trials on flue-cured tobacco, Burley tobacco, cotton, and on root-knot-free sweet-potato sprouts. However, in one test on heavily-galled sweet-potato sprouts, *F. oxysporum* produced symptoms indistinguishable from those caused by sweet potato or tobacco collections. *F. solani* (Mart.) App. and Wr. v. *eumartii* (Carp.) Wr. (Syn. *F. eumartii*) caused no symptoms of wilt on flue-cured tobacco, Burley tobacco, sweet potato, or cotton, but both types of tobacco were stunted and had slightly yellowed leaves. Examination showed stem-rot symptoms similar to those ascribed to *F. tobacivorum* Delac. (6). A second test with the original culture and a reisolation from stem

lesions on Burley tobacco gave similar results. The symptoms differed from "sore shin" of tobacco caused by *Rhizoctonia* sp. in that the lesion was near the base of the stem well below the soil surface and in the area where the larger roots emerged. It is possible that Delacroix worked with a *Fusarium* similar to *F. solani eumartii*.

## DISCUSSION

The limitations of host range tests based exclusively on artificial inoculations have been pointed out by the senior writer (15). It was shown that inoculation by artificial methods was not always a reliable method for determining susceptibility to a soil-borne disease. Heavy inoculation with the parasite and its byproducts combined with mechanical injury involved in the operation might break down the mechanism of resistance in plants. In that case the results obtained would not agree with disease occurrence under field conditions. The pathogenicity studies here reported are supported by field observations of an unusual type. The greater part of the observations, those made at Creedmoor and McCullers, North Carolina, and Tifton, Georgia, were made on plots of crop-rotation experiments conducted under controlled conditions to study other diseases. Hence, observations at these locations have the same weight as results obtained in experiments planned specifically for the study of the relation of preceding crop to the occurrence of fusarium wilt of tobacco. The observations of Armstrong (1) were made under less rigidly controlled conditions but are of much value because the tobacco on which wilt occurred was grown in areas where there was no record of the previous culture of this crop. In a study of this type, the writers feel that the accuracy of the inoculation technique is determined by whether the results of pathogenicity trials agree with occurrence of the disease under field conditions. The field observations demonstrated two relationships. First, the occurrence of fusarium wilt of flue-cured tobacco was associated with rotations of sweet potato. Second, the occurrence of *Fusarium* wilt of Burley tobacco was associated with rotations of cotton. Inoculation trials confirmed these two observations by demonstrating that certain isolates of Fusaria from sweet potato caused wilt of flue-cured tobacco and that all isolates from cotton caused wilt of Burley tobacco.

Cross-inoculations with Fusaria from flue-cured tobacco, Burley tobacco, cotton and sweet potato demonstrated that their host range over-lapped (Fig. 2). For example, collections from tobacco were always pathogenic on another plant, either sweet potato or cotton but not on both. Collections from sweet potato were always pathogenic on Burley tobacco, sometimes pathogenic on flue-cured tobacco and never pathogenic on cotton. Collections from cotton were consistently pathogenic on Burley tobacco, mildly pathogenic or negative on flue-cured tobacco, but negative on sweet potato. The wide variability of Fusaria of section Elegans with respect to microscopic and cultural characteristics has been demonstrated by many investigators (12, 14, 17, and others), but pathogenicity within the same "species" was thought to be much more constant. However, the data reported here

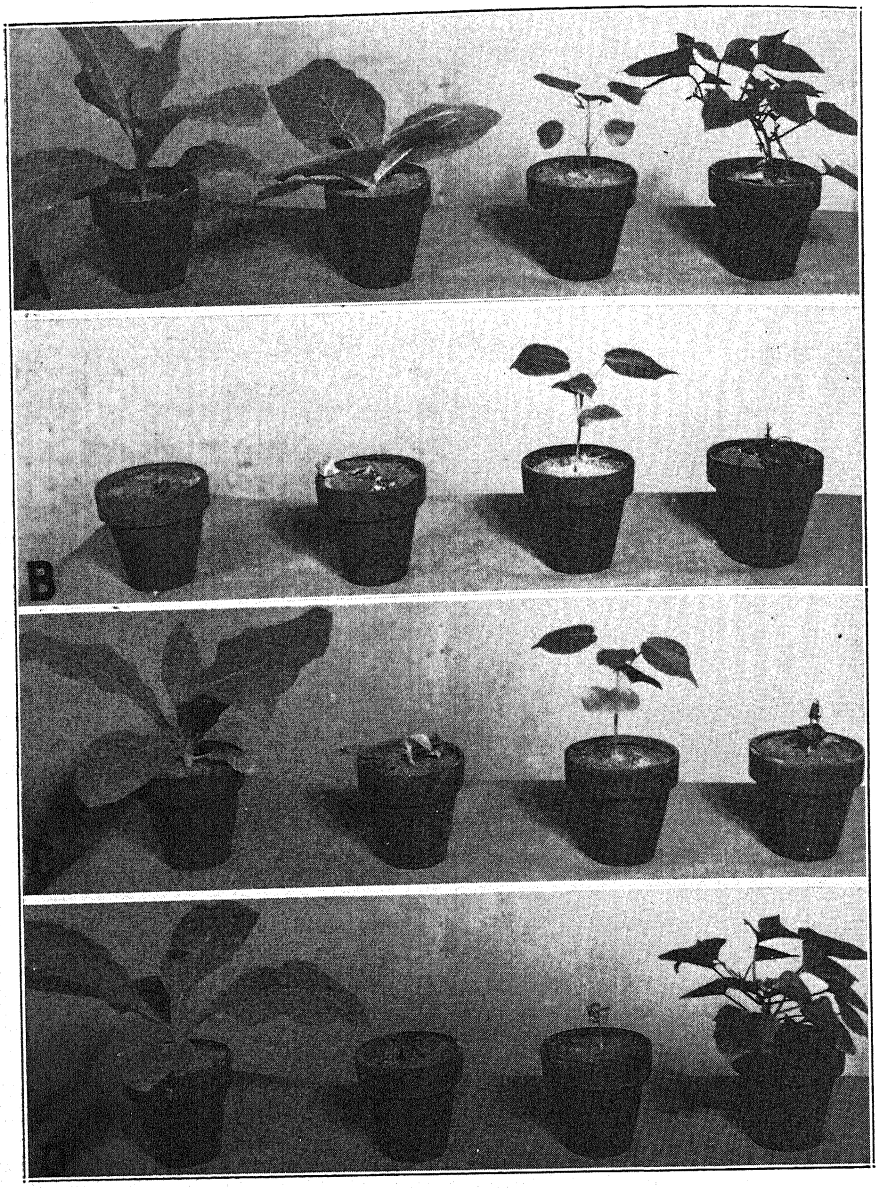



FIG. 2. Pathogenicity of Fusaria from tobacco, sweet potato, and cotton. Left to right: Flue-cured tobacco, Burley tobacco, cotton, and sweet potato. A. Noninoculated controls. B. Isolate from flue-cured tobacco. C. Isolate from sweet potato. D. Isolate from cotton.

and the work of Hansford (9) and Armstrong *et al.* (3)[4] and others show that pathogenicity may not be restricted to the plant species from which a race was isolated.

[4] Since the preparation of this manuscript the investigations of Armstrong and associates have been published in Phytopath. 32: 685–698. 1942.

A reasonable supposition about the origin of the various "species and varieties" of the Elegans section would be that there is one species, considered perhaps as *F. oxysporum* Schl., of more or less universal presence in the soil. It would then follow that various pathogenic races have developed whenever susceptible plants were grown for an adequate period of time. These races may or may not be pathogenically restricted to a single plant species; hence, in order to show the virulence of a single isolate for more than one host, a system based on physiologic races has some merit and requires consideration. For example, 93 of the 94 collections from tobacco, sweet potato and cotton can be separated into 3 physiologic races by the results of cross inoculations on these plants as shown in table 4. The one

TABLE 4.—*Summary of inoculation trials with vascular Fusaria isolated from tobacco, sweet potato, and cotton*

| Physiologic race number | Number of isolates obtained from | Results of inoculations on | | | |
|---|---|---|---|---|---|
| | | Flue-cured tobacco | Burley tobacco | Sweet potato | Cotton |
| | Sweet potato | | | | |
| 1 | 14 | − | + | + | − |
| 2 | 8 | + | + | + | − |
| | Cotton | | | | |
| 3 | 19 | − | + | − | + |
| | Flue-cured tobacco | | | | |
| 2 | 20 | + | + | + | − |
| 3 | 1 | − | + | − | + |
| | Maryland tobacco | | | | |
| 2 | 7 | + | + | + | − |
| 3 | 1 | − | + | − | + |
| | Burley tobacco | | | | |
| 1 | 1 | − | + | + | − |
| 2 | 11 | + | + | + | − |
| 3 | 6 | − | + | − | + |
| | Dark tobaccos | | | | |
| 2 | 1 | + | + | + | − |
| 3 | 4 | − | + | − | + |
| 3? | 1 | − | − | − | + |

collection not conforming to this system was Johnson's culture, 1937, from dark-fire-cured tobacco in Simpson County, Kentucky. This collection could be considered as race 3 when originally isolated but as having lost virulence for Burley tobacco while kept in storage; therefore, all 94 collections could be said to conform. Many problems, however, such as standardization of inoculation technique, maintenance of virulent cultures, and standardization of genetic resistance of test plants, must be worked out before a system based on physiologic races could be used successfully. Pathogenicity should be the major criterion for the classification of wilt-producing Fusaria, as

pointed out by several workers. Snyder and Hansen (12) proposed a new system based on assumed pathogenicity for single plant species. Wilt of sweet potato was attributed to *Fusarium oxysporum* f. *batatas*, wilt of tobacco to *F. oxysporum* f. *nicotianae* and wilt of cotton to *F. oxysporum* f. *vasinfectum*. Pathogenicity is not that uniform, as shown in table 4.

In considering the practical application of these results, the main conclusions have been verified by field observations and appear to be well founded. It is pointed out that fusarium wilt of tobacco is caused by races of the fungus virulent for sweet potato, cotton, and perhaps other plant species. The use of crop rotations combining sweet potato and all types of tobacco is a hazardous practice because both crops are susceptible to a common race of the parasite. In areas where the disease is present on flue-cured tobacco this combination of crops is exceptionally hazardous because nearly all of the collections from this type of tobacco attacked sweet potato. Fields have been observed in the Whiteville-Chadbourn area of North Carolina in which successive crops of flue-cured tobacco and sweet potato were badly damaged by fusarium wilt. The use of rotations combining cotton and flue-cured tobacco appears to be a safe practice even though most collections from cotton (table 3) were able, to a limited degree, to parasitize this type of tobacco. Burley tobacco was susceptible to all collections from sweet potato and cotton. Therefore, this type of tobacco would probably be subject to fusarium wilt at most locations throughout the Southeast, as observed by Armstrong (1), who found wilt in 6 of 8 fields examined. The situation on Maryland tobacco was not extensively surveyed, but all Maryland collections were virulent for flue-cured tobacco and sweet potato, while one collection made in the cotton belt at Florence, South Carolina, was virulent for cotton. It appears, then, that fusarium wilt of all types of tobacco may be caused by a race of the fungus pathogenic on sweet potato, but on Burley and dark-fired tobaccos it is also caused, in many instances, by a different race of the fungus, which also is pathogenic on cotton.

## SUMMARY

Field observations suggested a close relation between Fusaria causing wilt of tobacco, sweet potato, and cotton.

Pathogenicity trials with 53 collections from tobacco showed that fusarium wilt of tobacco in these cases was caused by races of the fungus virulent on sweet potato or cotton but not on both. The race pathogenic on sweet potato was obtained in nearly all collections in North Carolina, South Carolina, and Georgia from flue-cured tobacco and in Maryland from Maryland tobacco. Races pathogenic on sweet potato or cotton were collected in approximately equal numbers in Kentucky from Burley and dark tobaccos.

Pathogenicity trials with 22 collections of *Fusarium* from sweet potato showed that all were pathogenic on Burley, some but not all pathogenic on flue-cured tobacco, and all negative on cotton.

Pathogenicity trials with 19 collections of *Fusarium* from cotton were all pathogenic on Burley tobacco, slightly pathogenic or negative on flue-cured tobacco, and negative on sweet potato.

All 94 collections could be grouped into 3 physiologic races on the basis of results of inoculation on flue-cured tobacco, Burley tobacco, sweet potato, and cotton. The data show that more emphasis should be given to pathogenicity in the classification of Fusaria of the Elegans section.

In conclusion, it is pointed out, that the use of crop rotations combining the growth of sweet potato and all types of tobacco is a hazardous practice. Both crops are susceptible to a wide-spread race of *Fusarium*.

The growth of Burley and dark tobaccos in rotation with cotton also appears to be a hazardous practice. However, flue-cured tobacco may be safely grown in rotations with cotton.

TOBACCO BRANCH STATION, OXFORD, NORTH CAROLINA,
AND
NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION,
RALEIGH, NORTH CAROLINA.

LITERATURE CITED

1. ARMSTRONG, G. M. Fusarium wilt on tobacco in Piedmont South Carolina. U. S. Dept. Agr., Bur. Pl. Ind., Pl. Dis. Rptr. 22: 326–327. 1938.
2. ————. Fusarium wilt of cotton and tobacco apparently caused by the same organism. (Abstract) Phytopath. 30: 1–2. 1940.
3. ————, B. S. HAWKINS, and C. C. BENNETT. Cross-inoculations with fusarium-wilt organisms. (Abstract) Phytopath. 32: 20–21. 1942.
4. BROWN, W. Studies in the genus Fusarium. V. General description of strains, together with a discussion of the principles at present adopted in the classification of Fusarium. Ann. Bot. 42: 285–304. 1928.
5. BUGNICOURT, P. Les Fusarium et Cylindrocarpon de l'Indochine. Encycl. Mycol. 11. 206 pp. 1939. (Paul Lechevalier, Paris).
6. DELACROIX, G. Recherches sur quelques maladies du tabac en France. Ann. Inst. Nat. Agron., 5: 141–232. 1906.
7. DOIDGE, ETHEL M. Some South African Fusaria. Bothalia 3: 331–483. 1938.
8. GOSS, R. W. Fusarium wilts of potato, their differentiation and the effect of environment on their occurrence. Amer. Potato Jour. 13: 171–180. 1936.
9. HANSFORD, C. G. Vascular diseases of Cotton in Uganda. E. Afr. Agr. Jour. 4: 279–282. 1940.
10. JOHNSON, J. Fusarium-wilt of tobacco. Jour. Agr. Res. [U.S.] 20: 515–535. 1921.
11. MCMURTREY, J. E. The effect of boron deficiency on the growth of tobacco plants in aerated and unaerated solutions. Jour. Agr. Res. [U.S.] 38: 371–380. 1929.
12. SNYDER, W. C., and H. N. HANSEN. The species concept in Fusarium. Amer. Jour. Bot. 27: 64–67. 1940.
13. REINKING, O. A. Fusaria inoculation experiments: Relationship of various species of Fusaria to wilt and Colorado disease of banana. Phytopath. 16: 371–392. 1926.
14. RIKER, REGINA S., and L. R. JONES. Fusarium strains in relation to wilt of China aster. Phytopath. 25: 733–747. 1935.
15. SMITH, T. E. Host range studies with *Bacterium sonalacearum*. Jour. Agr. Res. [U.S.] 59: 429–440. 1939.
16. WELLMAN, F. L. A technique for studying host resistance and pathogenicity in tomato fusarium wilt. Phytopath. 29: 945–956. 1939.
17. ————, and DOROTHY J. BLAISDELL. Differences in growth characters and pathogenicity of Fusarium wilt isolations tested on three tomato varieties. U. S. Dept. Agr. Tech. Bull. 705. 28 p. 1940.

# A BROWNING REACTION TO STEM RUST IN WHEAT[1]

HELEN HART AND J. LEWIS ALLISON

(Accepted for publication September 7, 1942)

Reaction to rust infection has always been considered a relatively stable character in a cereal variety, and yet there may be considerable variability in abundance of sporulation, degree of chlorosis of host tissues, extent of mycelial development within tissues, and in the pigmentation surrounding the rust pustule or point of infection. In some instances the variability in reaction of certain wheat varieties to *Puccinia graminis tritici* Eriks. and Henn. is so extreme as to make difficult a placement of reaction type in one of the five classes described by Stakman and Levine (15). Sporulation may vary greatly (Fig. 1, A and C), and pigmentation around the point of infection (Fig. 1, A, B, and C) may be so dark as to distract attention from the usual slight chlorosis of host tissues (Fig. 1, D). Such a lack of sporulation and a deep pigmentation seem to indicate greater resistance to rust; and the present paper describes the frequency of this particular type of reaction to stem rust, the factors responsible for it, and its histological aspects.

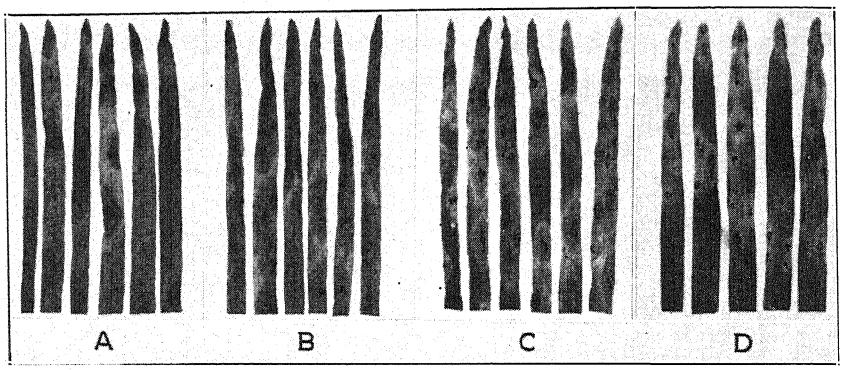



FIG. 1. Variability in reaction of Thatcher wheat to race 34 of *Puccinia graminis tritici*. A. Severe browning about point of infection and a trace of sporulation. B. Moderate browning and fair sporulation. C. Moderate browning and good sporulation. D. Normal reaction without browning, but with slight chlorosis about pustules.

## FREQUENCY OF BROWNING IN CERTAIN WHEAT VARIETIES

This reaction, which conveniently may be called a browning reaction, because the deep brown appearance of tissues about the point of infection is its outstanding characteristic, occurs rather often in seedlings of certain

[1] Published as Paper 2026 of the Journal Series of the Minnesota Agricultural Experiment Station.

Assistance in the completion of this work was furnished by the personnel of the Work Projects Administration, Official Project 165–71–1–124, sponsored by University of Minnesota.

The writers wish to thank F. S. Thatcher for photographing the material in figure 1, A, B, C; and Thomas A. Graham for the photomicrographs in figure 3.

wheat varieties, and seems never to occur in others. Vernal emmer, Arnautka, Kubanka, Kota, and Kanred are subject to the browning reaction; and selections from them or their hybrids often have the same reaction. This is true for Acme, a selection from Kubanka, and for the following hard red spring wheat varieties derived by hybridization from parental material subject to browning: Ceres, Rival, Reliance, Thatcher, Hope, and Pilot. The browning reaction may be frequent in the 14-chromosome *Triticum timopheevi* Zhuk, and in einkorn, as well as in the 14- and 21-chromosome species of *Triticum*. On the other hand, the reaction never has been observed in Marquis or in Little Club. Nevertheless, the browning reaction cannot be considered a varietal character, because its expression depends on the race of rust involved and the external environmental conditions incident to rust infection.

### ASSOCIATION OF THE BROWNING REACTION WITH CERTAIN RUST RACES

During the 3 or 4 years that the browning reaction was observed closely, certain physiologic races of *Puccinia graminis tritici* were associated with its occurrence more often than others (Table 1). Whenever collections of

TABLE 1.—*Host–parasite combinations in which the browning reaction frequently occurs in different varieties of wheat*

| Race of *Puccinia graminis tritici* | Susceptible host variety | Resistant host variety |
|---|---|---|
| 11 | ..... | Vernal |
| 15A | Acme, Arnautka, einkorn, Hope, Kota, Kubanka, Thatcher, Vernal | Khapli |
| 15B | Acme, Arnautka, einkorn, Kota, Kubanka, Thatcher | ..... |
| 21 | Ceres | ..... |
| 34 | Acme, Arnautka, Ceres, Hope, Kanred, Kota, Kubanka, Thatcher, Rival, Pilot | einkorn, Khapli, Vernal, *Triticum timopheevi* |
| 38 | Acme, Arnautka, Kubanka | Khapli |
| 40 | ..... | einkorn |
| 42 | Khapli | ..... |
| 56 | ..... | einkorn, Arnautka, Vernal |
| 79 | ..... | Arnautka |
| 147 | Kubanka | ..... |
| 166? | ..... | Arnautka, Mindum, Spelmar |

race 34 were cultured in the greenhouse, the browning was frequent and present in a large proportion of the differential *Triticum* varieties inoculated: at least 10 different collections of race 34, from various parts of the United States and from Scotland apparently induced the browning reaction in a selected lot of varieties under favorable environmental conditions. Race 34, whether collected from wheat or from barberry, seemed capable of inducing this particular variation of a normal stem rust reaction in more different wheat hosts and in more experimental instances than any of the other rust races tested.

The browning reaction was rather frequent in certain hosts rusted by a race 15 collected from Japan (a biotype designated 15A by Loegering and Stakman (10)) in 1942. Fewer hosts responded thus to the Japanese collection than to race 34, but incidence of browning over a period of many months approximated that with race 34. In later experiments another collection of race 15, corresponding to the biotype 15B of Loegering and Stakman (10) induced browning as effectively as did the Japanese collection.

When other races of stem rust were involved, fewer varieties had the browning reaction and its frequency in different experiments was lower. On several occasions infected tissues of Vernal emmer were deeply pigmented when rusted by a collection of race 11 from Chile. Kubanka seedlings sometimes reacted thus when rusted by races 38 or 147, and einkorn responded likewise to race 40. Other isolated instances of this variation in reaction occurred in response to races 21, 42, and 56; but it was never observed in any host rusted by races 17, 36, 59, or 69. In all circumstances, too, it was evident that environment played as great a role as did the cereal hosts and rust races concerned in the reaction.

### TEMPERATURE AS THE PRIMARY ENVIRONMENTAL FACTOR RESPONSIBLE FOR BROWNING

High temperature, above 28.5° C., was the one factor of the environment directly responsible for browning in certain wheat varieties inoculated with rust. Furthermore, the degree of pigmentation varied directly with the length of time the temperature remained above 28.5° C. Brief exposure of inoculated and infected seedlings to high temperature was sufficient to initiate the reaction, so that a slight browning was evident to the eye within a few days of the exposure. A deeper host coloring and reduced sporulation of the rust fungus followed longer and repeated exposures. If temperatures were high during the incubation period, within 2 to 4 days after inoculation, and before pustules were formed, the pigmentation was severe and fairly extensive, and there was less sporulation or it was almost inhibited (Fig. 1, A). Later exposure to high temperature, after pustule formation, seldom brought about a severe reaction, unless the exposure was prolonged.

Moderately high atmospheric humidity accelerates the expression of browning reaction, but it cannot induce the reaction without the primary stimulus of high temperature. Other environmental factors—light, nutrient relations, soil moisture—produced no noticeable effects. They were relatively unimportant when compared with temperature and with atmospheric humidity.

Since the browning seemed to indicate greater resistance to stem rust at high temperatures, the infected tissues were examined for indications of internal tissue changes or changes in development of the parasite.

HISTOLOGY OF THE REACTION

Numerous materials were prepared and sectioned in several different ways. The most satisfactory was the simplest: sectioning fresh material on the freezing microtome and mounting sections in lactophenol without staining. The pigments were retained better with such treatment than when material was fixed and killed, dehydrated with alcohols, and embedded in paraffin, then stained subsequent to sectioning.

A great abundance of mycelium was formed in most of the lesions, so that hyphae permeated the host tissues for some distance beyond the rust pustule. In the browned area of most hosts (Fig. 2) most of the rust haustoria had become dark and thick-walled and were so numerous as to account for a large part of the browning evident in a surface view of the

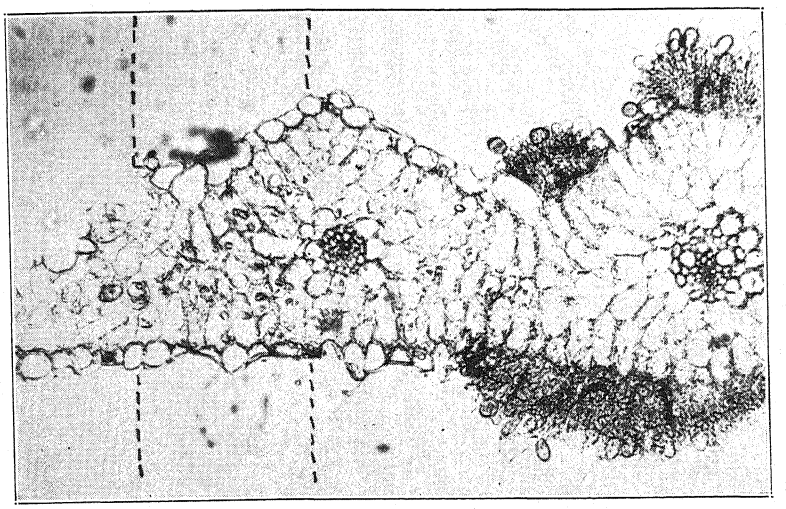


FIG. 2. Cross section of an Arnautka seedling with a moderate browning reaction to race 34 of *Puccinia graminis tritici*, showing an abundance of mycelium throughout the leaf tissue and a number of transformed haustoria (in the area between the dotted lines) at some distance from the rust pustule but coinciding with the browned area seen in a surface view of the leaf.

leaf. While the transformed haustoria were the most conspicuous features of the browned area they were not solely responsible for browning. Near the outer margins for mycelial development the cell walls were discolored in groups of host cells, usually a group of 5 to 15 cells not far removed from a vascular strand in the leaf. Host cell walls were brown and slightly thickened in many cases. If the sections were run through the alcohols the discolored walls were far less evident, and it was only from the appearance of freshly cut sections in lactophenol that we could judge how important the wall discolorations were in enhancing the "browning" effect seen in surface view of the leaf. One other thing contributed to the effect, the fact that the tips of some of the intercellular rust hyphae were discolored and sometimes transformed and ensheathed as were the haustoria. In a

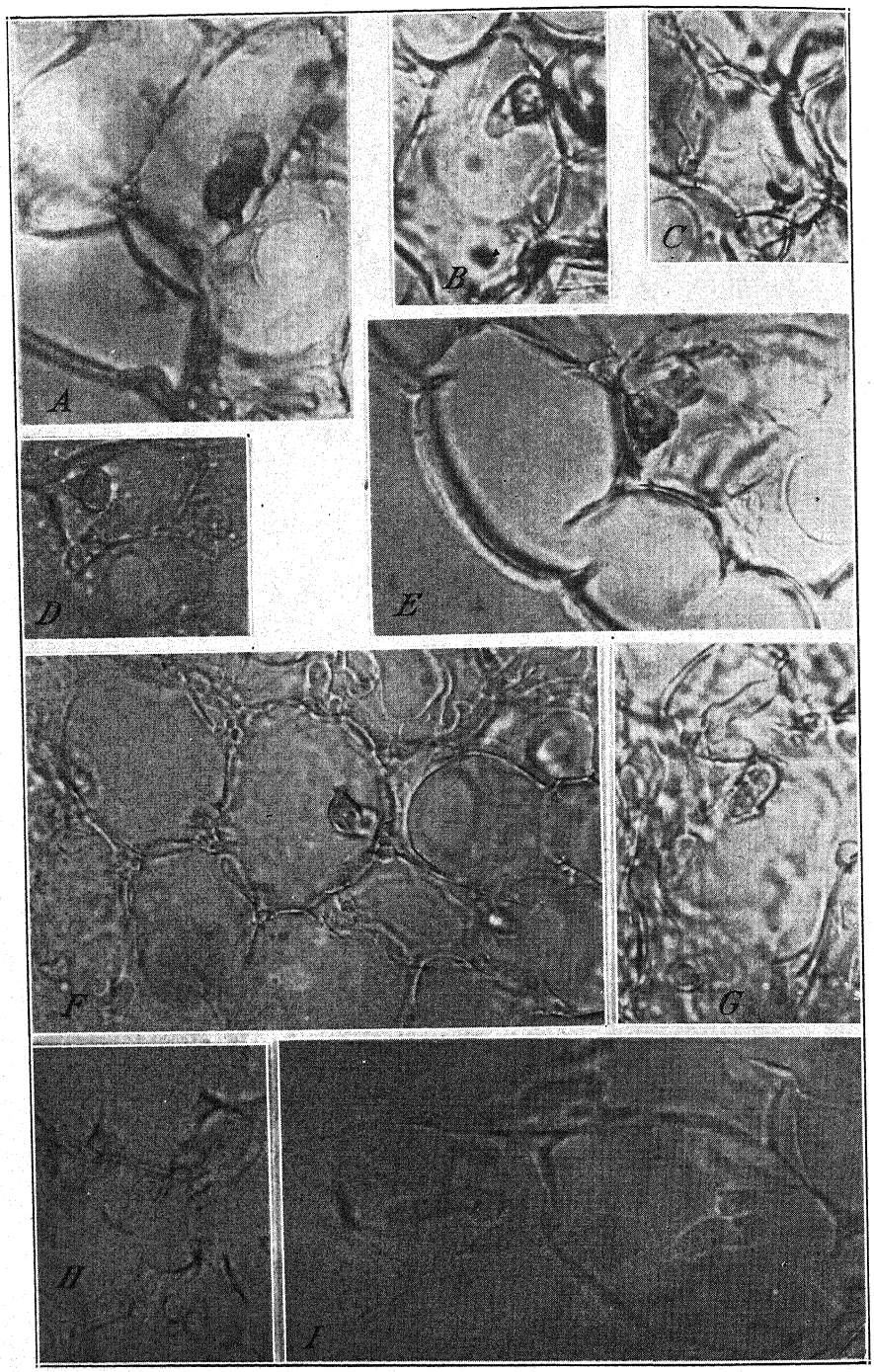



FIG. 3. Photomicrographs showing histological details associated with the browning reaction of wheats to *Puccinia graminis tritici*. *A*. Two haustoria of race 34 encysted within a host cell of Kubanka wheat, one completely encased in a thickened and darkened

few cases these hyphae were primarily responsible for the browning and were conspicuous in tissues immediately adjacent to the vascular bundles.

Studying the transformed haustoria more closely we saw that there had been an attempt at encystment, with results similar in many respects to what has been described by other authors (1 through 6, 12 through 14, and 16) for rust infections in a resistant host tissue or in an aging rust infection. In a cross section of a wheat seedling susceptible to stem rust the haustoria usually are delicate, elongate, sac-like protrusions into the host cell and host protoplast, connected to the intercellular rust hyphae by slender strands of protoplasm (Fig. 3, *I*). These normal haustoria also were present in seedlings with a browning reaction, but few in number in comparison with those that were partly to completely ensheathed. The formation of the thick sheath began at the base of the haustorium and about its neck, the slender protoplasmic thread joining the body of the haustorium to its mother cell (Fig. 3, *C* and Fig. 4, *D*). The sheath gradually enclosed most or all of the haustorium (Fig. 3, *B* and Fig. 4, *E* and *G*), but never was observed about the haustorium mother cell.

Pigmentation of the sheath varied considerably: it was sometimes dark brown to black in the collar-like sheaths that were formed early (Fig. 3, *C*) or in a completely encased haustorium (Fig. 3, *A*); it might be dark brown near the base of the haustorium body and less intense in the other parts (Fig. 4, *D, F,* and *G*); or it might be lacking in a part or all of the enclosing sheath (Fig. 4, *E* and Fig. 3, *G* and *H*). In a few sections there were occasional haustoria of exactly the same golden brown as the normal urediospores in nearby pustules in the same sections (See Fig. 4, *I*).

Ensheathed haustoria varied greatly in shape, very few of them being so elongate as normal haustoria unless the sheath itself was limited or slow in its development (Fig. 4, *D* and *E*). Some were nearly spherical (Fig. 3, *A* and *D;* Fig. 4, *F*), especially in cases of hosts resistant to the rust race present, or in cases of rapid and severe browning. By far the greatest number were ovoid, with a definite constriction at the middle (Fig. 3, *B*,

membrane, the other only partially encased. Haustorial mother cells were not affected. *B.* A single haustorium of race 15A partially encysted within a host cell of Acme wheat. Thickening has not yet occurred about the tip of the haustorium. *C.* An early stage in the sheathing of an haustorium of race 15A in Acme wheat. The very thick and dark saucer-shape sheath started at the base of the haustorium and around the slender strand of protoplasm between haustorium and its mother cell, but the tip of the haustorium was not changed. *D.* An encysted haustorium that was nearly spherical, a form encountered often when a resistant host (einkorn) had a browning reaction to race 34. *E.* An abortive teliospore formed by a hyphal tip of race 34 just beneath the epidermis of Kubanka wheat. Two cells were evident, the tip cell having the thick, dark, cap-like structure of a normal teliospore. *F.* Teliospore-like haustorium of race 15A associated with browning of Kubanka wheat. The tip of the haustorium had been encased in a thick, dark, papillate sheath similar to the wall of a teliospore, and the middle of the haustorium seemed to be constricted. The base of the haustorium, corresponding to the basal cell of a teliospore, was not in focus in the photograph. *G* and *H.* Urediospore-like haustoria of race 15A in cells of Acme wheat. The thick sheaths about the haustoria were not deeply pigmented but were rough and resembled the echinulate walls of normal urediospores. The spore mother cell and the hypha shown in B were not affected and had smooth walls. *I.* Normal thin-walled haustoria of race 34 in host cells of Kubanka seedlings that did not have a browning reaction.

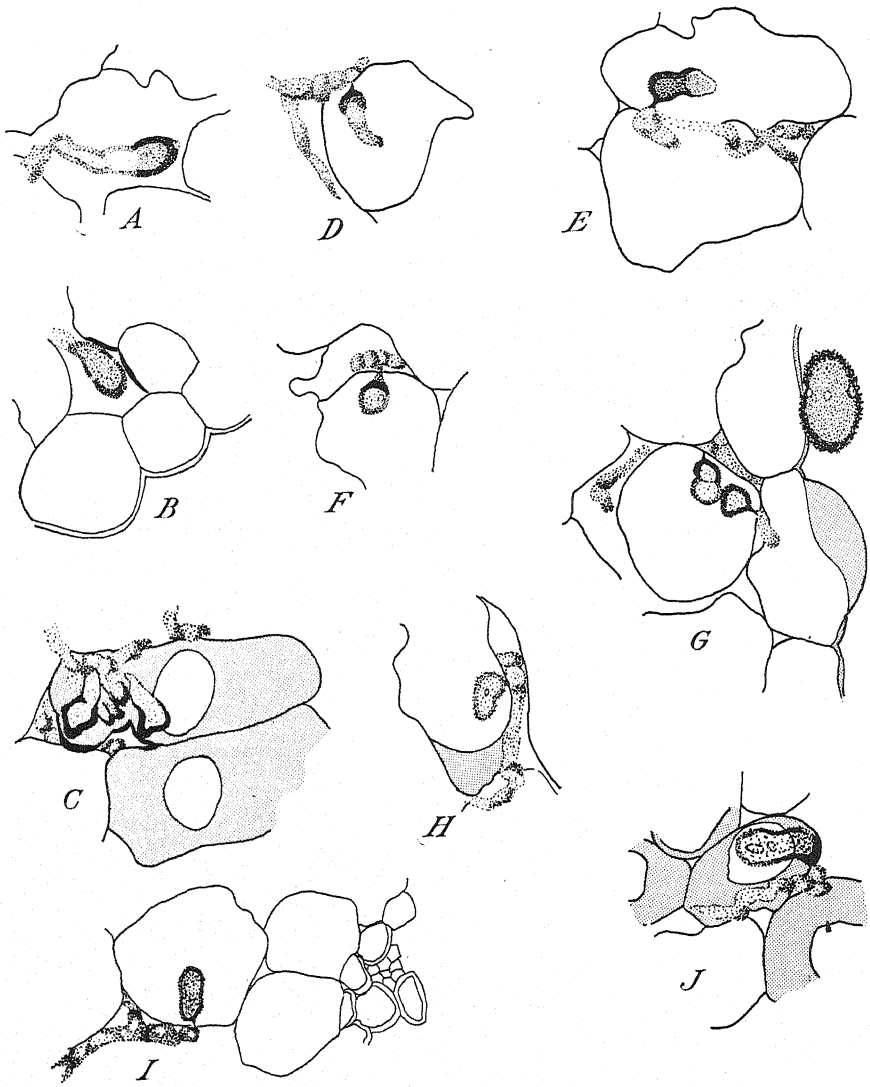



FIG. 4. Camera-lucida drawings of histological details associated with the browning reaction of wheat to *Puccinia graminis tritici*. *A*. Tip of a hypha of rust race 15A in an intercellular space in a Kubanka seedling. Constrictions in the hypha and its thick sheath with dark pigment and papilla at the tip gave it the appearance of an abortive teliospore. *B*. Hyphal tip of race 15A with thick, dark sheath in an intercellular space just beneath the epidermis of an Acme seedling. Host cell walls adjacent to the hyphal tip were somewhat thickened and dark. *C*. A mass of hyphae of race 34 lying on a host cell of Arnautka wheat. Two of the hyphal tips were thick and dark, slightly constricted, and somewhat papillate, so that they resembled abortive teliospores. Host cell walls near the transformed hyphal tips were thickened and dark. *D*. An early stage in the tranformation of an haustorium of race 15A in Kubanka wheat. Thickening and darkening of the sheath began at the base of the haustorium, while its tip was not affected until later. *E*. A later stage in the transformation of an haustorium of race 34 in Kubanka wheat. A thick sheath encased the entire haustorium, but pigmentation was evident over only $\frac{3}{4}$ of the sheath. *F*. A spherical, encysted haustorium of race 15A in a cell of Kubanka wheat, a type frequent in cases of severe browning. *G*. Two encysted haustoria of race 34 within a host cell of Kubanka. One haustorium resembled an abortive teliospore and appeared to

*F, G, H,* and Fig. 4, *E, G, H, I, J*), so that they called to mind abortive urediospores or teliospores on a miniature scale.

The sheath was smooth about most of the haustoria; but if pigment was lacking, or present in only a portion of the thick sheath, there appeared to be echinulations or a verrucose roughening of the sheath surface (Fig. 3, *G, H,* and Fig. 4, *H, J*). On rare occasions in a sheath almost devoid of pigment there were thinner areas in the sheath resembling in placement and proportionate area the germ pores of normal urediospores. Unfortunately, such cases were rare, and it was not possible to secure a photograph of such an haustorium.

The many similarities between ensheathed haustoria and rust urediospores or teliospores—golden to dark brown in lactophenol, shape and constriction about the middle, character of wall markings, and indications of thin areas—lead us to think that the haustorial sheath is a fungus rather than a host deposition. How long an ensheathed haustorium remains alive and how sheathing affects the permeability of the haustorial membrane and the subsequent nutrition of the rust parasite remain to be explained, and some research along these lines has been undertaken by F. S. Thatcher. We have been unable to separate an ensheathed haustorium from its host cell, and, so far as we can tell, there is no evidence that the haustorium, once it is ensheathed, ever resumes growth when favorable conditions return. We do not know that it actually is an abortive spore.

The tips of many of the intercellular rust hyphae had undergone changes similar to those in the haustoria within the cells. The hyphal membranes were greatly thickened, particularly at the very apex of a hypha, so that there was a papillate tip resembling that of a teliospore of stem rust (Fig. 3, *E;* Fig. 4, *A* and *C*). Pigment often was unevenly distributed in the thickened membrane (Fig. 3, *E;* Fig. 4, *A, B,* and *C*), but the greatest amounts were usually near the apical end. The swollen hyphal tip generally was constricted once or twice at short distances from its apex (Fig. 4, *A* and *C*). Sometimes a septum divided the swollen tip (Fig. 3, *E*). Again, the resemblance to teliospores was striking, except in the matter of size and in completeness of development. Host cell walls adjacent to such transformed hyphal tips sometimes were slightly thickened and discolored (Fig. 4, *B* and *C*), but this was not always true. The intercellular spaces immediately beneath the epidermis or contiguous to a vascular bundle were more often occupied by these changed hyphal tips than were the spaces in other parts of the leaf mesophyll, but there was never such an aggregation of hyphae as one might expect for the formation of a normal uredium or telium.

---

be divided by a cross membrane. Size of haustorium may be compared with the normal urediospore lying outside the epidermis of the host. *H.* Haustorium of race 15A with thick, rough wall, resembling an abortive urediospore more than a teliospore. Pigmentation was not especially noticeable. *I.* Urediospore-like haustorium of race 34 on Pilot wheat. In lacto-phenol this haustorium was golden brown as were the normal urediospores. *J.* Extremely large haustorium of race 34 in Kubanka wheat, with thick, dark sheath.

OCCURRENCE OF BROWNING IN OLDER PLANTS

The browning reaction has been observed in older plants of many of the varieties included in the experiments with seedlings. Such observations, however, were usually incidental to other experimental arrangements and results. In the few sections cut from older materials the histological features corresponded with those found in seedlings of the same varieties.

The senior writer (8) first called attention to deep brown or black pigmentation and restricted sporulation in plants of Hope wheat inoculated with race 21 of *Puccinia graminis tritici* and kept at high light intensities or at high temperatures. Unfortunately, less attention was paid at that time to the histological study of early stages of infection, and in practically all of the material prepared from late stages of infection there were many dead host cells and dense mycelial infections that failed to sporulate or that produced pustules closely confined within the host tissues and with abortive and distorted spores. The rust collection used at that time is no longer available and it has been impossible to check and extend the previous work. It is believed, however, that the phenomenon encountered at high light intensity and high temperature is similar to but more severe than that herein described.

Similar pigmentation and discoloration were described by McFadden (11) for Hope and related wheats inoculated with races 11, 17, and 21 of *Puccinia graminis tritici* and grown in field plots in Texas in 1936 and 1937. McFadden uses the term "brown necrosis," because he found many of the plant cells were killed whenever the discolored blotches appeared. As early as 1915 he observed the reaction in Vernal and Yaroslav emmers, and later on Acme durum also. He reported no histological studies and no work with seedlings.

Johnson and Hagborg (9) also studied discolorations in wheat and found that some were caused by stem rust. Races 34 and 120 of *Puccinia graminis tritici,* particularly the latter, caused brown necrosis on heads and peduncles of Apex wheat. The discolorations were observed in varieties derived from crosses of Marquis and Reward with Hope, H 44, and Pentad. In glumes of Renown wheat examined histologically the stem rust had formed appressoria and entered the stomata but very little mycelium had developed beyond the substomatal cavity and the parenchyma cells appeared to be highly hypersensitive to rust. These authors also reported no work with seedlings.

It is noteworthy, however, that these browning reactions in relation to stem rust infection are most conspicuous by their presence in the hot and dry decade 1930–1940. Observations of Hart and Zaleski (8) covered the years 1932 and 1933; the extensive observations of McFadden (11) were made in 1936 and 1937; and Johnson and Hagborg (9) reported discolorations generally prevalent at Winnipeg on glumes and lemmas of many newly developed wheat varieties in 1935 and 1938; and the research herein reported was begun in 1936 and completed in 1940. From conversations

with E. C. Stakman, it also is apparent that this type of reaction was frequent during the identification of physiologic races of wheat stem rust collected during the physiologic-race survey of 1934, a year marked by exceptionally high temperatures in Minnesota. The durum wheats in the different series, especially Acme and Arnautka, often were less susceptible than expected. Race 34 of *Puccinia graminis tritici* generally was reisolated from poorly sporulating and off-color pustules on Acme and from the seemingly resistant reaction types on Arnautka. In 1934, when race 34 constituted 22 per cent of the stem rust races identified from collections on wheat, and often during hot periods of later years, the browning action might easily have been used as a supplementary means of identification when race 34 was mixed with certain other races in a collection. Whether browning will be of general occurrence on a number of hosts in the future remains to be seen.

## GERMINATION OF UREDIOSPORES AT HIGH TEMPERATURE AND IN HOST PLANT EXTRACTS

Since race 34 of *Puccinia graminis tritici* was more often associated with browning than any of the other races, we tested the effect of high temperature on the germination of its urediospores to note any tendency toward encystment of the fungus outside the host plant when urediospores germinated in distilled water or in extracts from certain hosts.

Plant sap was expressed from healthy, 7-day-old, Kubanka seedlings that had been frozen quickly at $-3°$ C. The sap was passed through a sintered glass filter and diluted with sterile distilled water to the desired concentration (1:100, 1:1000, 1:10,000, 1:100,000, and 1:1,000,000). Checks were in hanging drops of sterile distilled water.

At 27° C. the germ tubes were long and sinuous and often branched, both in distilled water and in the filtrates. At 33° C., however, a large proportion of the germ tubes grew to a length of 2 or 3 times the urediospore length but then rounded up to form a bulbous portion (Fig. 5, *B* and *C*). After a short time the germ tube resumed growth from a pore-like opening in the bulbous encysted cell. Such abnormal germ tubes were fairly abundant in all dilutions of the filtrates and in distilled water at 33° C. Frequently the tips of longer germ tubes were transformed to spore-like bodies at the high temperature. In figure 5, *A*, a germ tube, 5 to 6 times the urediospore length, had a tip with a very thick wall, dark-brown pigment in the thickened wall, a papillate thickening at the apex, and what appeared to be two separate cells. Resemblance to an abortive teliospore is very pronounced. Ezekiel (7) found structures of the same sort produced by germinating urediospores of several different races of *Puccinia graminis tritici* and also other rusts.

## DISCUSSION

The browning reaction well illustrates the possibility of rather wide variations in reaction to stem rust. While in resistant hosts the browning

reaction is only an added indication of resistance, in a susceptible host a severe browning reaction may signify a definite shift from susceptibility to resistance.

The results of such variations may be more far-reaching than would seem evident from limited experimental work. It is possible that such a variation as this browning reaction has been one of the factors responsible for the fact that race 34 no longer plays an important part in the stem rust epidemics in the Mississippi Valley. In 9 of the 12 years from 1930 to 1941, inclusive, race 34 constituted less than 5 per cent of the stem rust population,[2] although many of the varieties of winter wheats, hard red

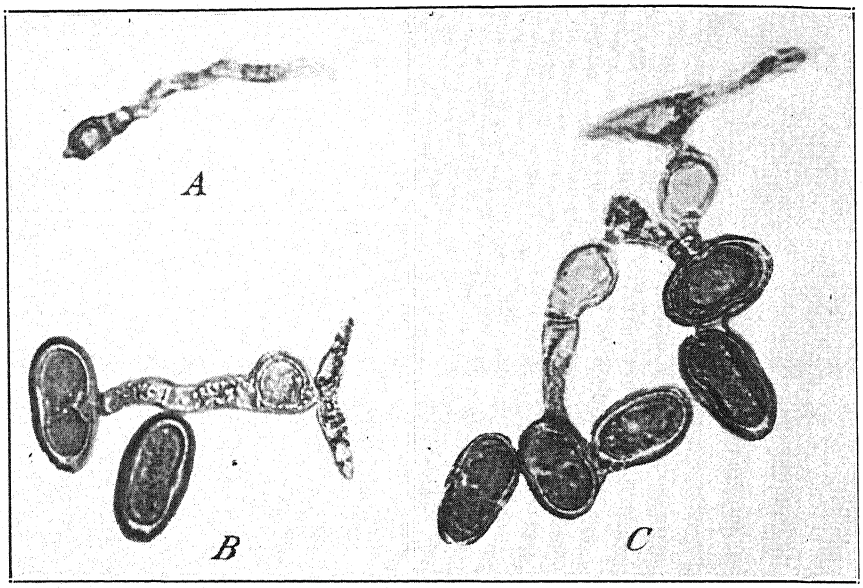



FIG. 5. Photomicrographs of the germination after 18 hours of race 34 of *Puccinia graminis tritici* at 33° C. in filtrates of 7-day-old Kubanka seedlings. *A*. Tip of a germ tube that produced an abortive teliospore, in concentrated filtrate. *B*. Germ tube from a urediospore, with swelling and an attempt at encystment in the third cell formed, and with renewed growth of a slender, branched, thin-walled germ tube from the swollen cell. In a 1: 1,000,000 filtrate. *C*. Two germ tubes in which the second cell formed had swollen and from the swollen cells new germ tubes had grown out. The swollen cells seemed to be empty of protoplasm. In a 1: 10,000 filtrate.

spring wheats, and also durums are susceptible to this race. In the spring wheat region race 34 survived the hot, dry years 1930 through 1934 as well as or better than many of the other stem rust races, but by 1934 race 56, which Cassell[3] found more tolerant of high temperatures than most of the other rust races, predominated. Since the entire decade 1930–1940 may

[2] We are indebted to E. C. Stakman and W. Q. Loegering, Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, for data from their unpublished reports.

[3] Cassell, R. C. Factors affecting the distribution of physiologic races of *Puccinia graminis tritici*, Eriks. and Henn. University of Minnesota. Ph.D. thesis, June 1938. (Unpublished.)

be characterized as hot and dry for the Mississippi Valley in general, it is not surprising that the heat-tolerant race 56 supplanted the other races to so great an extent. Race 34 was not immediately eliminated with the rapid rise of race 56, however, and in 1934 and again in 1935 there were surprisingly large proportions of race 34—22 per cent and 18 per cent, respectively. The explanations for the persistence of race 34 lie in the environment during those years and in the earliness with which rust developed. In 1934 stem rust appeared 7 to 10 days earlier than usual; and early in a growing season when temperatures usually are low and moisture is sufficient race 34 undoubtedly has as good a chance for survival as other races. With 7 to 10 days start in 1934 race 34 might have multiplied enough to become an important factor in the stem rust epidemic. Watson (17) found that race 34 was a good competitor when grown in association with other races on Little Club or on a Soft Federation wheat from Australia. It is true that the incubation period for race 34 on certain susceptible hosts seems from $\frac{1}{2}$ to 2 days shorter than for other races. Such an advantage undoubtedly contributed to the build-up of early season inoculum of race 34. The spring of the following year, in which race 34 was important, was cold and wet; cloudly weather prevailed until the end of June. Even after hot weather set in towards the end of June, there were frequent showers to modify the high temperatures and to contribute to stem-rust development in plants that may have been inoculated during the cooler, cloudy days of mid-June.

The fact that many of the new rust-resistant hybrids and much of the breeding material seem subject to browning may help in further reduction of certain races in the future rust populations if we have more growing seasons marked by extreme heat. If, however, we have several seasons of moderate temperature we might experience an increase of certain races involved in browning reactions, provided susceptible hosts are at hand for their increase.

Study of the browning reaction also brings up many questions as to adjustments of the obligately parasitic rust fungi to their gramineous hosts, and some of these were previously raised in Rice's (12, 13) excellent articles. It is hard to say whether the haustorium is actually the specialized structure we have heretofore considered so characteristic of obligate parasites or whether it is somewhat more like the normal intercellular rust hyphae. Both seem capable of the same reactions under the adverse conditions described in the present work. The indications here are that the sheathing of the haustoria or the hyphae occurs by the same process that brings about normal sporulation of the rust fungus. It is still not known whether changes in permeability, shifts in oxidation relationships, increases in the activity of certain other enzymic systems, or other specific metabolic changes may provoke sporulation or encystment of the fungus. And in cases of normal sporulation, as well as of protective sheathing, we cannot tell what determines whether urediospores and urediospore-like bodies will be produced or teliospores and teliospore-like bodies will appear.

SUMMARY

The browning reaction consists of a deep brown discoloration of wheat host tissues about a stem rust infection center, usually accompanied by reduced sporulation of the rust.

Browning has been associated with only certain wheat varieties infected with certain races of stem rust and under certain environments.

Temperatures above 28.5° C. constitute the primary environmental factor responsible for browning. High relative humidity enhances the effects of high temperature.

Rust hyphae and rust haustoria become ensheathed and transformed. Resemblance of the transformed parts to urediospores and teliospores is striking in several respects: pigmentation, shape and constriction about the middle, character of wall markings, indications of a band of thin areas in the wall, and papillate apical thickenings.

Host cell walls in a browned area may be discolored and slightly thickened.

There is some tendency toward spore-like encystment of germ tubes when urediospores of race 34 of *Puccinia graminis tritici* germinate at high temperature.

UNIVERSITY FARM,
ST. PAUL, MINN.

LITERATURE CITED

1. ALLEN, RUTH F. A cytological study of infection of Baart and Kanred wheats by *Puccinia graminis tritici*. Jour. Agr. Res. [U. S.] 23: 131–152. 1923.
2. ————. Cytological studies of infection of Baart, Kanred, and Mindum wheats by *Puccinia graminis tritici* forms III and XIX. Jour. Agr. Res. [U. S.] 26: 571–604. 1924.
3. ————. Cytological studies on forms 9, 21, and 27 of *Puccinia graminis tritici* on Khapli emmer. Jour. Agr. Res. [U. S.] 32: 701–725. 1926.
4. ————. A cytological study of *Puccinia triticina* physiologic form 11 on Little Club wheat. Jour. Agr. Res. [U. S.] 33: 201–222. 1926.
5. ————. A cytological study of orange leaf rust, *Puccinia triticina* physiologic form 11, on Malakoff wheat. Jour. Agr. Res. [U. S.] 34: 697–714. 1927.
6. ————. A cytological study of *Puccinia glumarum* on *Bromus marginatus* and *Triticum vulgare*. Jour. Agr. Res. [U. S.] 36: 487–513. 1928.
7. EZEKIEL, W. N. Studies on the nature of physiologic resistance to *Puccinia graminis tritici*. Minn. Agr. Exp. Stat., Tech. Bull. 67. 1930.
8. HART, HELEN, and KAROL ZALESKI. The effect of light intensity and temperature on infection of Hope wheat by *Puccinia graminis tritici*. Phytopath. 25: 1041–1066. 1935.
9. JOHNSON, T., and W. A. F. HAGBORG. Brown necrosis and Alternaria blotch of wheat. Sci. Agr. 22: 746–760. 1942.
10. LOEGERING, WM. Q., and E. C. STAKMAN. Biotypes within *Puccinia graminis tritici*, race 15. (Abstract.) Phytopath. 32: 12–13. 1942.
11. MCFADDEN, E. S. Brown necrosis, a discoloration associated with rust infection in certain rust-resistant wheats. Jour. Agr. Res. [U. S.] 58: 805–819. 1939.
12. MOSS, E. H. The uredo stage of the Pucciniastreae. Ann. Bot. 40: 813–847. 1926.
13. RICE, MABEL A. The haustoria of certain rusts and the relation between host and pathogen. Bull. Torr. Bot. Club 54: 63–153. 1927.
14. ————. The cytology of host-parasite relations. Bot. Rev. 1: 327–354. 1935.
15. STAKMAN, E. C., and M. N. LEVINE. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Minn. Agr. Exp. Stat., Tech. Bull. 8. 1922.
16. TAI, F. L. Studies on *Juniperus chinensis*. I. *Gymposporangium yamadae* Miyabe. Lingnan Sci. Jour. 9: 13–28. 1930.
17. WATSON, I. A The development of physiological races of *Puccinia graminis tritici* singly and in association with others. Proc. Linnean Soc. New So. Wales 67: 294–312. 1942.

# PHYSICAL CHARACTERISTICS OF BORDEAUX MIXTURE IN RELATION TO ITS QUALITIES

E. E. WILSON

(Accepted for publication September 1, 1942)

The common belief among plant pathologists that the physical characteristics of the precipitate of Bordeaux mixture influence the fungicidal value of the spray is typified in the following statement by Lutman (8). "The peculiar physical structure of the copper compound which is precipitated is the characteristic which gives to Bordeaux mixture a great share of its value. . . ."

According to a number of investigators (1, 2, 5, 7, 11) the concentration of the constituents at the time of mixing influences the suspension qualities of the precipitate. If, in making 8–8–100 Bordeaux, for example, the copper sulphate and lime in stock preparations, which commonly contain from 0.5 to 1 lb. of constituent to each gallon of water, are first mixed and then diluted with the requisite amount of water, the resulting precipitate settles more rapidly than that formed when the constituents are each diluted with one-half the required amount of water and then mixed. A difference in rate of settling, therefore, indicates the physical characteristics, in this case the specific gravity, of the precipitate.

From studying formation of precipitates under different conditions, Swingle (10) concluded that the combination of relatively concentrated preparations produced more of the "closed cells"—spherical sacs of lime suspension surrounded by membranes of the blue precipitate—than the combination of more dilute preparations. Lutman (8) came to similar conclusions, particularly with reference to the effect of a concentrated copper sulphate solution. He reported that, when undisturbed, the membranes surrounding closed cells thickened until considerable force was required to break them, and that rate of thickening was greatest when a relatively concentrated copper sulphate solution was used.

Swingle (10) believed that the precipitate of a properly prepared Bordeaux mixture contained much imbibed water, a condition that would contribute to its bulkiness. In certain aspects this has been confirmed by Reckendorfer (9), who found that the precipitate swelled measurably in water. The ratio of lime to copper sulphate, furthermore, affected the swelling capacity, inasmuch as increasing the lime to copper sulphate above 1:1 was accompanied by a decrease in swelling.

Lutman (8) believed that agitation broke the precipitation membranes which surround closed cells, and aided in the reaction between the copper sulphate and lime, and thus affected both the quantity and thickness of precipitation membranes. Hawkins (5) showed a definite increase in suspension qualities when Bordeaux prepared from a dilute copper sulphate solution and a concentrated lime suspension was shaken vigorously. In such

instances one of the primary functions of agitation is preventing the local concentration of one constituent by quickly dispersing it throughout the larger volume of liquid containing the other.

Although settling rate and to some extent volume of the precipitate have been used to indicate quality of Bordeaux (1, 5, 6, 7, 11), search of the literature shows but few instances where attempts were made to determine the relation between the physical characteristics of the precipitate and either its general fungicidal performance or the factors that influence this performance, *i.e.*, the toxicity of the precipitate to fungus spores, amount and distribution of deposit over the sprayed surface, or resistance (tenacity) of the deposit to removal by rain. Without giving his evidence Cunningham (3) asserted that only by mixing the two constituents in a concentrated form did he obtain "unsatisfactory results." Under greenhouse conditions Yarwood (12) found but little difference between the immediate protective and eradicant values of Bordeaux mixtures prepared from concentrated and diluted stock preparations of copper sulphate and lime, when applied in control of powdery and downy mildews of a number of plants. In the case of bean rust, however, the mixture prepared from diluted preparations was somewhat superior to that prepared from concentrated preparations.

The primary purpose of this article is to report studies on the weather resistance of Bordeaux mixtures that varied in physical characteristics because of differences in method of combining copper sulphate and lime. Information of this type is particularly pertinent to effective prevention of a peach and apricot disease caused by the fungus *Coryneum beijerinckii*. The fungus attacks twigs and dormant buds in winter. To prevent this infection an application of Bordeaux, 10–10–100, is given in the autumn subsequent to leaf fall. As this application is expected to protect the buds and twigs throughout the winter (14 to 16 weeks) when heavy rains occur, the tenacity with which the deposit resists removal determines to no small degree the control obtained.

Other factors, such as the fineness and the calcium carbonate content of the lime, are known to influence the suspension qualities of Bordeaux mixture (4, 6), but the relation of these to tenacity of the deposit was not studied in the present work.

## SETTLING, DEPOSITION, AND TENACITY OF BORDEAUX MIXTURE

The writer's studies on the tenacity of Bordeaux mixtures prepared from diluted and concentrated constituents were conducted in the field in December, 1941, and January, 1942. The trees used for the purpose were 4-to-5-year-old peaches and apricots of uniform size. The surfaces on which the tenacity of the spray was determined were those of twigs produced in 1941 only.

After the spray was applied, at a pressure of 300 pounds, and had thoroughly dried, 3 samples of twigs, 4–6 inches long, were taken from each

tree. These were weighed immediately and placed in glass jars with tight-fitting glass tops. Two hundred cc. of nitric acid solution (25 cc. of C.P. nitric acid, sp. gr. 1.416, per liter) was run into each jar, the tops fixed in place, and the jars rolled for 10 minutes on a mechanical rolling device. The solution was then filtered and aliquots were taken for copper determination by the iodometric method.[1]

In test 1, the Bordeaux made with diluted constituents, and hereafter known as mixture 1, was prepared by pouring a suspension containing 1.6 lb. of slaked quicklime into the spray tank containing 15 gal. of water. With the agitator running 1 gal. of a solution containing 1.6 lb. of copper sulphate was then poured in and the required amount of water to make 20 gallons was added. After 5 minutes' agitation,[2] samples of the settling tests were taken of the mixture in the tank and of the mixture issuing from the nozzle of the spray gun. The spray was then applied.

In test 1, the Bordeaux made with concentrated constituents, and hereafter known as mixture 2, was prepared from the same lots of copper sulphate and lime used above, but instead of diluting one lot, the solution containing 1.6 lb. of copper sulphate per gal. was poured into the suspension containing 1.6 lb. of slaked quicklime per gal. The resulting mixture was stirred thoroughly and poured into the sprayer tank containing the required amount of water to make 20 gallons. After 5 min. agitation, samples for settling tests were taken from the tank and spray nozzle. The spray was then applied.

In tests 2 and 3, mixture 1 was made by slowly pouring 2 lb. of powdered copper sulphate into the sprayer tank containing 18 gal. of water. When the copper sulphate was dissolved, 2 lb. of hydrated lime, thoroughly mixed with 2 gal. of water a few minutes earlier, was poured into the tank while the agitator was running. After agitating 5 minutes, samples for settling tests were taken from spray nozzle and tank, and the spray was then applied.

In tests 2 and 3, mixture 2 was prepared as was mixture 2 in test 1, except that hydrated lime was used. After 5 minutes' agitation, settling-test samples were taken from tank and nozzle. Three trees were then immediately sprayed. The remaining Bordeaux mixture in the tank was agitated for an extra 15 minutes (a total of 35 minutes) and a settling-test sample was taken from the nozzle before it was applied to two other peach trees.

The results of copper analyses are contained in table 1, the results of settling tests for test 1 and 3 are given in figure 1. Settling-test data for test 2 were essentially the same as those for test 3.

## Rate of Settling

According to figure 1, mixture 1, taken from the sprayer tank after 5 minutes' agitation, settled very little during 75 minutes, whereas mixture 2

[1] This method is satisfactory for relatively large amounts of copper if the nitric acid is free of nitrous oxide and determinations are made on the wash water immediately after it is filtered.

[2] In this and the other tests the samples of Bordeaux taken from the sprayer tank had not circulated through the pump, whereas that taken from the nozzle of the spray gun had, of course, passed through both pump and nozzle.

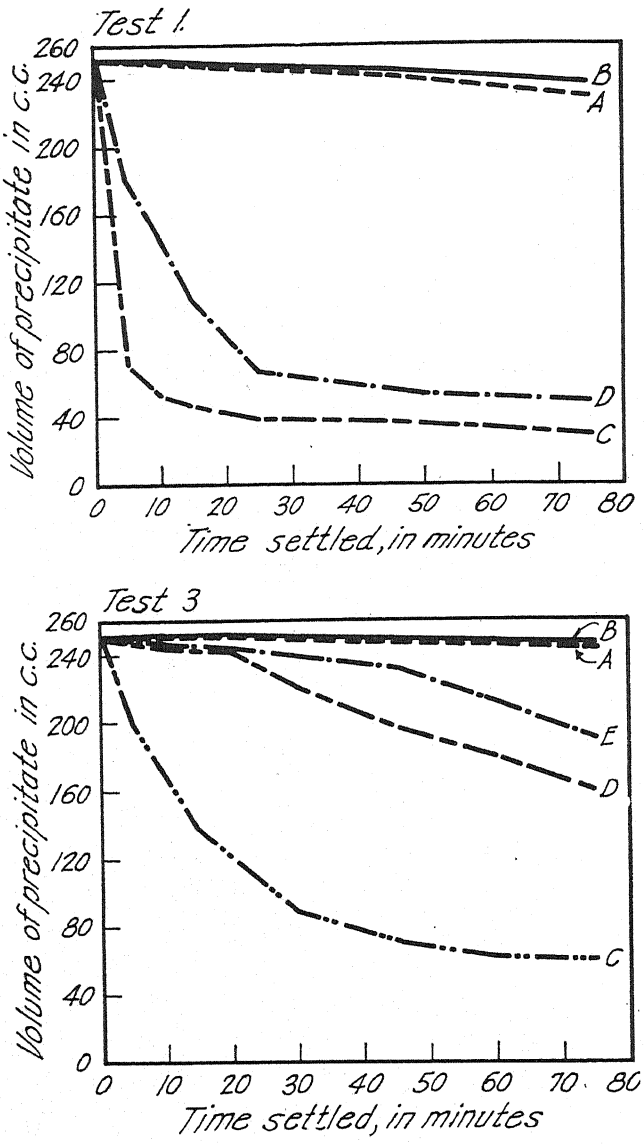



FIG. 1. Effect of method of mixing and the character of agitation on the settling of Bordeaux precipitate. Test 1: A, mixture 1, prepared from diluted lime and concentrated copper sulphate, agitated 5 min. in sprayer tank; B, mixture 1, agitated 5 min. then passed through pump and spray nozzle; C, mixture 2, prepared from concentrated copper sulphate and concentrated lime, agitated 5 min. in sprayer tank; D, mixture 2, agitated 5 min. in sprayer tank, passed through pump and spray nozzle. Test 3: A, mixture 1, prepared from diluted copper sulphate and concentrated lime, agitated 5 min. in sprayer tank; B, mixture 1, agitated 5 min. in sprayer tank then passed through pump and spray nozzle; C, mixture 2, prepared from concentrated copper sulphate and concentrated lime, agitated 5 minutes in sprayer tank; D, mixture 2, agitated 5 minutes in sprayer tank then passed through pump and spray nozzle; E, mixture 2, agitated 35 minutes in sprayer tank then passed through pump and spray nozzle.

from the tank settled very rapidly. In fact, as shown by observations after 18 hours, the latter mixture had settled almost completely within 75 minutes. Mixture 2, which, in addition to 5 minutes' agitation, had gone through the pump and issued from the nozzle of the spray gun, settled somewhat more slowly than that taken from the tank. This was particularly noticeable in test 3. Prolonging agitation of mixture 2 for 35 minutes resulted in a still more slowly settling precipitate.

After settling 18 hours the bulkiness of the precipitate was observed to differ among the Bordeaux mixtures made by the two methods. The volumes (cc.) occupied by the precipitates in test 1 were as follows: Mixture 1 from tank, 72; mixture 1 from nozzle, 103; mixture 2 from tank, 31; mixture 2 from nozzle, 37. With mixture 1 the volume of precipitate was increased by passing through the pump and spray nozzle, whereas mixture 2 was affected little if any.

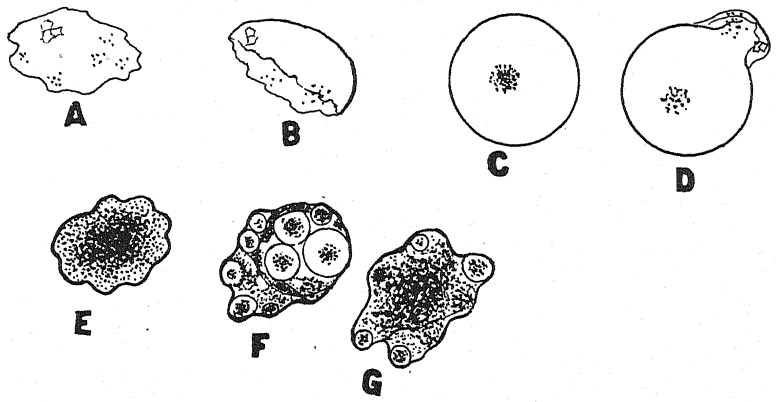



FIG. 2. Characteristics of the particles of precipitate formed when the following concentrations of copper sulphate and lime were mixed: A, limewater and 0.17 per cent copper sulphate; B, both at 0.25 per cent; C–D, both at 0.5, 1, and 2 per cent; E–G, both at 12 per cent.

Judging from the settling tests, the method of diluting one of the constituents (lime in the case of test 1 and copper sulphate in the case of test 3) resulted in precipitates with suspension qualities that could not have been materially excelled by that produced had both constituents been diluted.

To determine what difference existed between the particles of precipitate in mixtures prepared from diluted and concentrated preparations, the following concentrations of $CuSO_4$ solution and lime suspension were mixed with a minimum of agitation: 1. 0.17 per cent lime and 0.168 per cent $CuSO_4$; 2. Both at 0.25 per cent; 3. Both at 0.5 per cent; 4. Both at 1 per cent; 5. Both at 2 per cent, and 6. Both at 12 per cent. Under the microscope the precipitate of No. 1 was composed largely of transparent flakes (Fig. 2, A) that of No. 2 contained a considerable portion of what appeared to be ruptured closed cells (Fig. 2, B). At concentrations of 0.5 to 2 per cent a considerable part of the precipitate was in the form of closed cells

TABLE 1.—*Effect of concentration of the two components, when mixed, on the tenacity of Bordeaux mixture on peach and apricot twigs. December to February 1941–1942*

| Test No.[a] | Method of mixing Bordeaux | Tree number and statistical references | Amount of copper on 100 g. of twigs | | Copper lost |
|---|---|---|---|---|---|
| | | | Initial deposit | After rain[b] | |
| | | | *Mg.* | *Mg.* | *Per cent* |
| 1 | Concentrated copper sulphate poured into diluted lime, agitated 5 minutes before applying. Mixture 1. | 1 | 17.4 | 15.2 | 12.6 |
| | | 2 | 16.8 | 12.4 | 26.2 |
| | | 3 | 20.0 | 16.9 | 15.5 |
| | | Average | **18.0** | **14.8** | **18.1** |
| | Concentrated copper sulphate poured into concentrated lime, agitated 5 minutes before applying. Mixture 2. | 1 | 22.5 | 7.8 | 65.3 |
| | | 2 | 19.5 | 7.3 | 62.6 |
| | | 3 | 21.2 | 6.8 | 67.8 |
| | | Average | **21.1** | **7.3** | **65.2** |
| | | Diff. for signif.[c] | *9.8* | *3.4* | *20.3* |
| 2 | Concentrated lime poured into diluted copper sulphate, agitated 5 minutes before applying. Mixture 1. | 1 | 37.7 | 24.9 | 34.0 |
| | | 2 | 34.5 | 24.1 | 30.1 |
| | | 3 | 37.2 | 23.4 | 37.1 |
| | | Average | **35.7** | **24.1** | **33.7** |
| | Concentrated copper sulphate poured into concentrated lime, agitated 5 minutes before applying. Mixture 2. | 1 | 26.8 | 7.7 | 71.3 |
| | | 2 | 31.0 | 10.2 | 67.1 |
| | | 3 | 29.7 | 8.0 | 73.1 |
| | | Average | **29.2** | **8.6** | **70.5** |
| | | Diff. for signif.[c] | *5.4* | *2.6* | *12.4* |
| 3 | Concentrated lime poured into diluted copper sulphate, agitated 5 minutes before applying. Mixture 1. | 1 | 32.4 | 21.3 | 34.3 |
| | | 2 | 38.2 | 23.2 | 39.3 |
| | | 3 | 31.2 | 19.0 | 39.1 |
| | | Average | **33.9** | **21.2** | **37.6** |
| | Concentrated copper sulphate poured into concentrated lime, agitated 5 minutes before applying. Mixture 2. | 1 | 19.0 | 7.6 | 60.0 |
| | | 2 | 22.8 | 11.8 | 48.3 |
| | | 3 | 26.5 | 9.5 | 64.2 |
| | | Average | **22.8** | **9.6** | **57.5** |
| | | Diff. for signif.[c] | *5.2* | *3.1* | *23.5*[d] |
| 3A | Concentrated copper sulphate poured into concentrated lime, agitated 35 minutes before applying. Mixture 2. | 1 | 26.9 | 12.2 | 54.7 |
| | | 2 | 29.2 | 12.1 | 58.6 |
| | | Average | **28.0** | **12.2** | **56.6** |

[a] In test 1 Bordeaux 8–8–100 was prepared with freshly slaked quicklime. In tests 2, 3, and 3A Bordeaux 10–10–100 was prepared with hydrated lime. Trees in tests 1, 3, and 3A were peaches, in test 2, apricots.

[b] The amount of rain falling between application and this analysis was: test 1, 4.12 inches; tests 2, 3, and 3A, 2.96 inches.

[c] The difference for significance calculated on the basis of *t* values for 99:1 odds.

[d] The difference between means exceed the value for 19:1 odds but not the value for 99:1 odds.

(Fig. 2, C, D). At 2 per cent (No. 5) the membranes surrounding the cells were less transparent than at 0.5 or 1 per cent. At 12 per cent the precipitate (No. 6) was composed of a large number of closed cells illustrated in figure 2, E to G. Some of these cells enclosed dense masses of lime and some enclosed smaller cells along with the lime.

Laboratory tests showed that, whereas such membranes as illustrated in figure 2, C, D, readily broke when the mixture was agitated, and some broke without agitation, those illustrated in 2, E to G did not break so readily. In fact the ordinary whirling motion afforded by a laboratory stirrer broke but few of these particles and had little or no effect on their suspension quality. The increase in suspension, shown above to result from passing mixture 2 through the pump and spray nozzle, may therefore have been because this violent agitation was sufficient to break the surrounding membranes and free the enclosed lime aggregates.

## Initial Deposits

In test 1 the initial deposits of the two types of Bordeaux did not differ significantly (Table 1, column 3) but in tests 2 and 3, mixture 1 deposited significantly more copper than mixture 2.

Despite the fact that the analysis showed that mixture 1 deposited as much or more copper than mixture 2 on peach trees, the deposit of the latter was more conspicuous than that of the former. This was because the deposit of mixture 2 was coarser and appeared to stand up from the surfaces, whereas that of mixture 1 was fine in texture and laid closely to the surface. These differences were apparent also when the sprays were deposited on glass slides.

## Tenacity of Deposits

In all tests (Table 1) mixture 1 weathered away less rapidly than mixture 2. In test 3 the difference between means of residue after rains and means of percentage of copper lost is significant at 19 : 1 odds but not at 99 : 1 odds. This was largely because the amount of residual copper on tree 2, sprayed with mixture 2, proved exceptionally high.

It will be noted that only 18.1 per cent of the copper in mixture 1 made with quicklime (Test 1) weathered away during rains totaling 4.12 inches, whereas 33.7 and 37.6 per cent, respectively, of that of mixture 1 made with hydrated lime (Tests 2 and 3) weathered away during rains amounting to 2.96 inches. This would suggest that the hydrated lime Bordeaux was less tenacious than quicklime Bordeaux, but should not be considered conclusive proof.

As noted earlier an increase in length of agitation period (Test 3, E) decreased the settling rate of mixture 2, and the deposit studies suggest that there was some increase in tenacity attending this additional agitation, but the data are too meager for conclusions, particularly in view of the large variation between the percentages of copper lost from one tree sprayed with mixture 2 in test 3.

Because uncombined lime gives to a Bordeaux deposit much of its visibility, disappearance of this lime may lead one to believe that the copper deposit also had been lost. Between the two types of deposits studied in these tests, however, such a wide difference in visibility existed after weathering had occurred, that no question arose as to the actual difference in

copper loss. On peach twigs, as noted earlier, the initial deposit of mixture 2, because of its coarse, granular nature, was much more conspicuous than the finer textured initial deposit of mixture 1; but, after weathering, the residue of mixture 2 was visible only around the bases of buds, whereas that of mixture 1 remained visible over all the twig surface.

## DISCUSSION

The results just given substantiate the general belief that the physical characteristics of Bordeaux precipitate, which are determined by the concentration of the copper sulphate and lime preparations, play an important role in determining the efficacy of this fungicide. The criterion of efficacy in these studies was the resistance of the copper deposit to washing off by rain (tenacity). The precautions against mixing concentrated, *i.e.*, 12 per cent, copper sulphate and lime, therefore, appear justified when maximum tenacity to such twig surfaces as those of peaches and apricots is desired.

Settling tests almost always show Bordeaux mixture, prepared by combining concentrated components, possesses poor suspension quality. Rapid settling can be decreased to some extent by agitation, particularly by passage of the mixture through the pump and nozzle, but, within the limits of practicability, it is doubtful if agitation can entirely overcome this feature. Agitation probably breaks the closed cells and disperses lime aggregates, but whether it has any effect on the nature of the membranes themselves is not known. The results of one test, suggesting that the tenacity of Bordeaux made with concentrated constituents was improved somewhat by prolonged agitation, are interesting but not conclusive.

The differences in texture of precipitates apparent, in the dried deposits of the two types of Bordeaux mixtures, suggests one reason for the low tenacity of the deposit of that made with concentrated, constituents. Being coarse, this deposit stands up from the surface and probably is more exposed to erosion by rain than the finer deposit of Bordeaux made with diluted constituents.

Effects of differences in mixing on the depositional qualities of the resulting mixtures were not conclusively proved, though in two of the three tests Bordeaux mixture prepared with diluted components deposited significantly more copper than Bordeaux mixture prepared with concentrated components. To thoroughly explore this subject, retention of the liquid phase and behavior of the solid phase during application should be studied carefully.

## SUMMARY AND CONCLUSIONS

Bordeaux precipitate made by mixing diluted components was settled more slowly and was more bulky than that made by mixing concentrated components (12 per cent) and then diluting. The individual particles of the precipitate of the former (components diluted) were nearly all fragments of closed cells that ruptured during the reaction between the components or

during the moderate agitation given in laboratory tests. The membranous fragments were thin, pliable, and possibly at a high state of hydration. The particles of the latter (components concentrated) were in many cases agglomerations of lime held together by closed membranes that did not easily rupture during the reaction and apparently were not easily broken by agitation.

In 3 weathering tests conducted on twigs of peach and apricot trees during the winter, when rainfall totaled 3 to 4 inches, Bordeaux, prepared with diluted components, lost only 18, 34, and 38 per cent of the copper, whereas the Bordeaux prepared with concentrated components lost 65, 70, and 58 per cent.

Vigorous and prolonged agitation improved the suspension quality of the concentrated type. One test suggested that the agitation possibly improved its weathering quality, but the differences were not great enough for statistical significance.

In two of three tests the Bordeaux made with diluted constituents deposited sigificantly more copper than that made with concentrated constituents. The initial deposit of the latter, however, was more visible on peach twigs because it was coarser in texture and stood up from the surface more than that of the former.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA.

## LITERATURE CITED

1. BUTLER, O. Bordeaux mixture: I. Physico-chemical studies. Phytopath. 4: 125–180. 1914.
2. ————, and J. O. SMITH. Relative adhesiveness of the copper fungicides. Phytopath. 9: 431–444. 1919.
3. CUNNINGHAM, G. H. Plant protection by the aid of therapeutants. 243 pp. J. McIndoe. (Dunedin, N. Z.) 1935.
4. DE ONG, E. R., and W. C. ROOT. The effect of calcium carbonate on Bordeaux mixture. Phytopath. 15: 183–186. 1925.
5. HAWKINS, L. A. Some factors influencing the efficiency of Bordeaux mixture. U. S. Dept. Agr. Bur. Pl. Ind. Bull. 265: 29 pp. 1912.
6. HOLLAND, E. B., C. O. DUNBAR, and G. M. GILLIGAN. Influence of form and proportion of lime used and method of mixing on the resulting Bordeaux mixture. Jour. Agr. Res. [U. S.] 34: 677–686. 1927.
7. JONES, L. R. II. Bordeaux mixture. I. Methods of preparing Bordeaux mixture. Vt. Agr. Exp. Sta. Ann. Rpt. (1895) 9: 88–92. 1896.
8. LUTMAN, B. F. The covering power of the precipitation membranes of Bordeaux mixture. Phytopath. 2: 32–41. 1912.
9. RECKENDORFER, P. Zur Physikochemie der Kupferkalkbrühe (Haftfähigkeit als Quellungserscheinung). Zeit. Pflkrankh. 45: 341–353. 1935.
10. SWINGLE, WALTER T. Bordeaux mixture: its chemistry, physical properties, and toxic effect on fungi and algae. U. S. Dept. Agr. Div. Veg. Phys. and Path. Bull. 9, 37 pp. 1896.
11. WARREN, G. F., and J. A. VOORHEES. Methods of making bordeaux mixture. N. J. Agr. Exp. Stat. Ann. Rpt. (1906) 27: 231–234. 1907.
12. YARWOOD, C. E. Fungicidal value of Bordeaux mixtures prepared from diluted and concentrated stock solutions. (Abstract) Phytopath. 32: 19–20. 1942.

# TESTS OF ERADICANT SPRAYS FOR USE AGAINST SCLEROTINIA LAXA AND CORYNEUM BEIJERINCKII IN APRICOTS AND ALMONDS

E. E. WILSON

(Accepted for publication September 1, 1942)

Two fungus diseases, the brown-rot blossom and twig blight (*Sclerotinia laxa* Ader. and Ruh.) and the shot-hole (*Coryneum baijerinckii* Oud.), are widespread and destructive in apricot and almond orchards in California. Brown-rot blossom blight recently was shown[1] to be measurably reduced by a dormant application of calcium arsenite[2] (2 or 3 lb. per 100 gal. of water). The object of spraying with this material is to prevent the production of the conidia-bearing mats (sporodochia) on blighted, hold-over twigs in the tree and to destroy the sporodochia already present. So far as known these blighted twigs and the occasional rotted fruits that hang in the tree are the only sources of inoculum for blossom infection in the spring. The mycelium of the fungus, surviving in these parts from one season to the next, develops the sporodochia, which break through to the surface and produce conidia in late winter. Shot hole affects buds of apricot (Fig. 1), the buds and twigs of almond in winter, and blossoms, leaves, and fruit of both in spring. Apparently, only conidia are produced by the causal fungus in California.[3] These are found in greater or less abundance on the various diseased organs, but the mycelium in diseased buds, twigs, and an occasional blighted blossom remaining in the tree, is the only known means whereby the fungus survives from season to season. Conidia form the year around within blighted buds, but apparently only in winter and spring on the surface of twig lesions. The problem of controlling this disease by eradicant means, therefore, is that of preventing the fungus from surviving or from producing spores in these host parts.

The calcium arsenite spray is being used in California for control of *Sclerotinia laxa* and with comparatively little injury to apricot trees. It cannot, however, be safely applied to almond and certain prune varieties. An alternate material, harmless to the trees and effective against both *S. laxa* and *Coryneum beijerinckii*, would, therefore, be of great value. The writer here reports tests in which a number of compounds were tested against both fungi.

## EFFECT OF VARIOUS MATERIALS ON PRODUCTION OF SPORODOCHIA OF SCLEROTINIA LAXA ON BLIGHTED TWIGS

Sodium dinitro-o-cresylate (Elgetol containing 30 per cent of this salt) was included in tests with calcium arsenite as early as 1939. In table 1 are

[1] Wilson, E. E. Experiments with arsenite sprays to eradicate *Sclerotinia laxa* in stone-fruit trees as a means of controlling the brown rot disease in blossoms. Jour. Agr. Res. [U.S.] 64: 561–594. 1942.

[2] Although called monocalcium arsenite in the article cited above, there are reasons for believing this is not the proper designation. In any event, the material is one of the less basic of the calcium arsenites.

[3] A recent search in infected, overwintered leaves and in diseased buds and twigs of almond revealed no other stage.

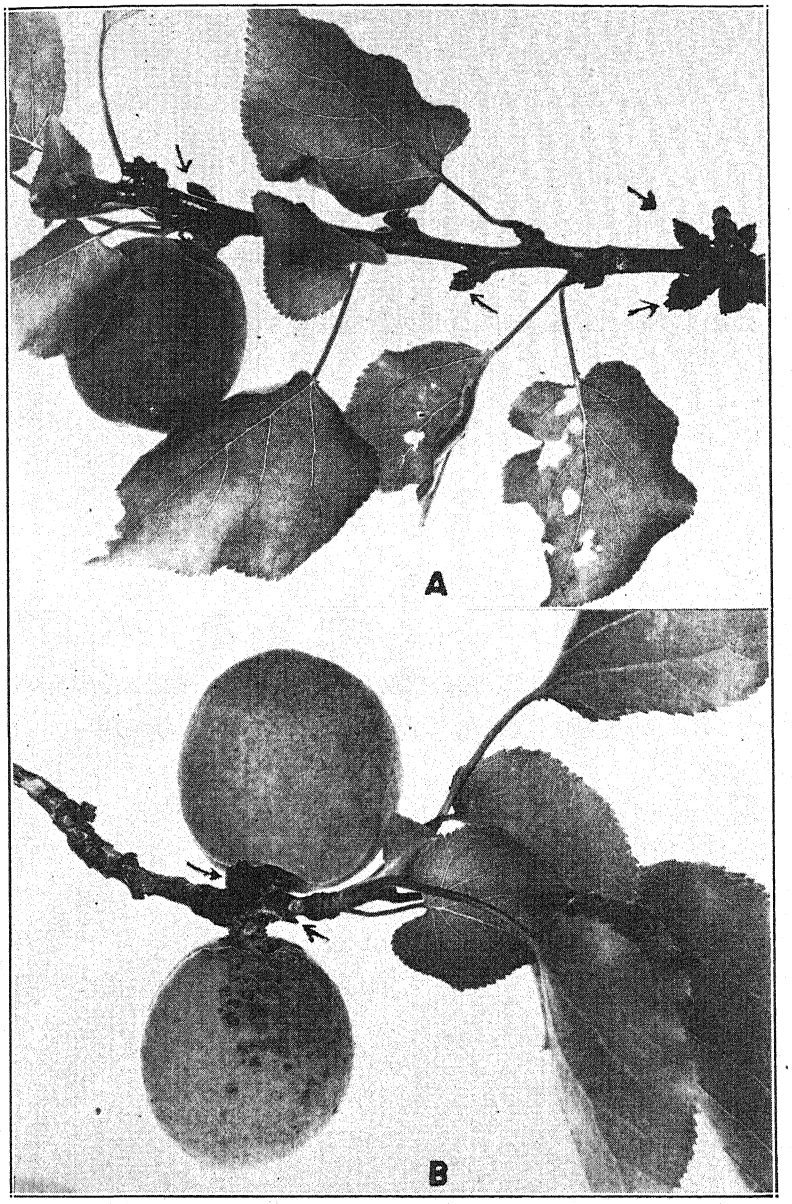



FIG. 1. Shot hole (*Coryneum beijerinckii*) affecting buds and fruit of apricot. A. Blighted buds (arrows) furnish conidia that infect leaves (infected leaves with holes) and fruit in spring. B. Fruit borne above blighted buds, healthy, whereas that below such buds, badly affected.

TABLE 1.—*Effect of spraying with dinitro-o-cresylates on the production of sporodochia by Sclerotinia laxa in apricot and almond trees during the winter*

| Kind of tree and year | Materials used and date of spraying | Reduction in the abundance of sporodochia on holdover twigs[a] | Reduction in the infection of blossoming shoots |
|---|---|---|---|
| | | *Per cent* | *Per cent* |
| Apricot, 1939 | Sodium dinitro-o-cresylate (Elgetol), 0.3 per cent, Jan. 18 | 95 | 78 |
| Almond, 1939 | Sodium dinitro-o-cresylate, 0.3 per cent, Jan. 17 | 81 | 12 |
| Prune, 1939 | Sodium dinitro-o-cresylate, 0.3 per cent, Dec. 15 | 53 | ..... |
| Almond, 1940 | Sodium dinitro-o-cresylate, 0.3 per cent, Feb. 1 | 92 | 68 |
| Apricot, 1941 | Sodium dinitro-o-cresylate, 0.3 per cent, Dec. 9 | 48 | ..... |
| | Sodium dinitro-o-cresylate, 0.3 per cent, plus ammonium sulphate, 0.5 per cent, Dec. 9[b] | 41 | ..... |
| Apricot, 1941 | Sodium dinitro-o-cresylate, 0.3 per cent, Feb. 4, 1941 | 80 | ..... |
| | Triethanolamine dinitro-o-cresylate, 0.3 per cent, Feb. 4 | 65 | ..... |
| Almond, 1942 | Sodium dinitro-o-cresylate, 0.3 per cent, Jan. 7 | 56 | 2 |

[a] Data taken at the end of bloom period.

[b] Ammonium dinitro-o-cresylate was presumably produced by adding ammonium sulphate to the sodium salt.

included all tests in which the object was to observe the effect of this material on production of the sporodochia by *Sclerotinia laxa*. These data show a great variation in the percentage reduction of sporodochia,[4] when the material was used at a strength of 0.3 per cent of the salt. A reduction of 92 and 95 per cent., respectively, accompanied its used in two tests, 80 and 81 per cent in two tests and 48, 53, and 56 per cent in three tests. A decrease in blossom blighting followed the reduction in sporodochial development, but was marked only where a high degree of suppression of sporodochia was obtained.

In one instance where ammonium sulphate was added to sodium dinitro-o-cresylate, thus producing the ammonium salt of dinitro-o-cresol, no difference in results followed. In one test triethanolamine dinitro-o-cresylate proved somewhat less effective than the sodium salt.

Furthermore, according to the data in figure 2, the suppressive effect of sodium dinitro-o-cresylate decreased between the beginning and the end of bloom. In 1939, for example, reduction in the number of sporodochia at the beginning of bloom was 93 per cent, at the end of bloom 53 per cent. In 1941 the percentages were 92 and 80, respectively, and in 1942, 80 and 56.

[4] The percentage of the diseased, holdover twigs that produced sporodochia and the average number of sporodochia per twig were determined on 200 randomly selected twigs from each treated and nontreated plot. The product of these two values provided index numbers from which the percentage reduction of sporodochia by the sprays was calculated.

These data, furthermore, indicate, as did those in table 1, that (with one exception in table 1) the suppression induced by February applications was greater than that following the December and January applications. In order to test this relationship further, small-scale applications on January 9 1942, were compared with those made on February 2, 1942. At the earlier date sporodochia had not appeared on the twigs, nor was there evidence that they were breaking through the twig periderm. At the latter date, however, sporodochia with conidia had appeared, and numerous others were breaking through the periderm. When applied at the time sporodochia were becoming exposed on the twig surfaces, the spray reduced their development by 77 per cent, but when applied before the sporodochia became exposed the reduction was only 39 per cent.

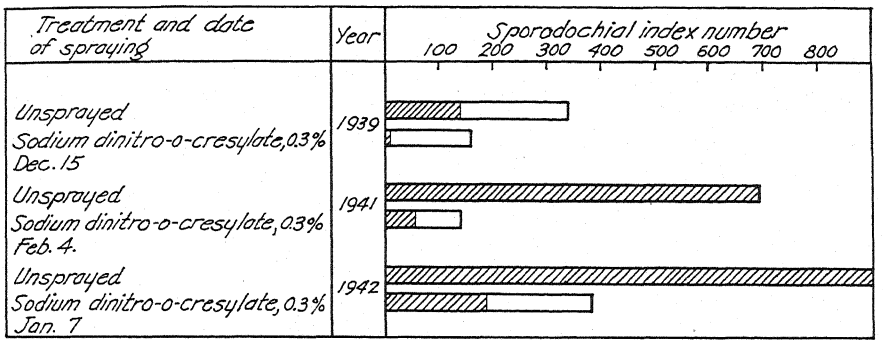



FIG. 2. Relative abundance of sporodochia at the beginning (cross bar) and at the end (cross bar plus white bar) of blossoming period, in nonsprayed apricot and almond trees and in similar trees sprayed in winter and in early spring with sodium dinitro-o-cresylate. The sporodochial index number is the product of the percentage of twigs with sporodochia and the average number of sporodochia per twig.

To determine the effect of the spray on the viability of the fungus within the tissues, twigs receiving sodium dinitro-o-cresylate and nontreated twigs were cultured. The number of sprayed and nonsprayed twigs with live mycelium differed but little, showing that the spray was not, under the conditions of these tests, killing the mycelium of the fungus embedded in host tissue. Apparently, therefore, the failure of the spray to kill embedded mycelium accounts for the development of new sporodochia between the beginning and end of bloom.

The suppressive effect of other materials also was tested in field studies between 1939 and 1942. These were tar oil-emulsion, 3 per cent; potassium ethyl xanthate, 1 per cent; sodium dimethyl-dithio-carbamate, 0.5 per cent; ferric dimethyl-dithio-carbamate, 0.5 per cent; an emulsible cresol, 1 per cent; zinc sulphate, 0.5 per cent; and ammonium sulphate, 0.5 per cent. None of these measurably reduced the development of sporodochia. In 2 tests of copper aceto-arsenite (Paris green) 0.5 per cent satisfactory suppression resulted, but in one test the results were unsatisfactory. This

material was objectionable in that it seriously injured almond trees in the 2 tests where suppression of sporodochia was most satisfactory.

### EFFECT OF VARIOUS MATERIALS ON SPORODOCHIA OF SCLEROTINIA LAXA

Attention was next directed to the effect of delaying the sprays until shortly before the blossoming period, when most of the sporodochia are exposed on the twig surfaces. If the spray destroyed most of the conidia on these structures, blossom infection might be lowered provided the mycelium in the twigs produced no new conidia before the trees were through blooming. Spraying cannot, however, be delayed much after the blossom buds begin to break, since the materials are known to injure the blossoms. Hence, in some seasons as much as two weeks will elapse between spraying and the beginning of bloom.

In order to test more materials, and replicate tests of the more promising ones, the following method was employed: Blighted, hold-over almond and apricot twigs bearing sporodochia were cut from the tree and stood upright in moist sand in 6-inch flower pots. They were then thoroughly sprayed with the desired material and after the spray dried were placed in a lath house where the conditions were essentially those of the outdoors except that strong winds were avoided. After a period of one week the spores from 10 to 15 sporodochia, picked at random from each group of sprayed and nonsprayed twigs, were suspended in separate lots of sterile, distilled water. The number of spores in each suspension was adjusted to about the same value by the addition of water, and drops of these suspensions were placed on the surface of each of several freshly poured plates of potato-dextrose agar. After 15 hours incubation at 25° C., the percentage germination of spores was determined. The conditions under which these experiments were conducted favored development of new sporodochia and conidia; so, by holding the twigs for a week after the germination tests, the effect of the materials on further development of these structures also was determined.

Forty-one materials were tested in 3 tests in February and March, 1941. The following at 1 per cent concentration neither destroyed the conidia nor prevented their further production: Paris green; ferric, sodium, and copper dimethyl-dithio-carbamates; sodium, copper, and lauryl thiocyanates; copper, zinc, and ammonium sulphates; an emulsible cresol; mercurochrome; salicylic acid; oxalic acid; hydroquinone; emulsions of paraformaldehyde, xylol, and paradichlorobenzene; an alcoholic solution of iodine; phenothiazine; calcium cresylate; calcium cyanamid; sodium chlorate; potassium permanganate; potassium dichromate; mercurous chloride; emulsified pine oil; and emulsified furfural. The following also were ineffective: 10 per cent lime-sulphur, 2 per cent tar oil-emulsion, 0.4 per cent ethyl mercury phosphate (Lignasan), and 0.25 per cent arsenic trioxide.

Six materials showed enough promise to be included in further trials. One per cent sodium tetrachloro-phenate, 1 per cent sodium pentachloro-

phenate, 0.45 per cent sodium dinitro-o-cresylate and 1 per cent sodium orthophenyl-phenate reduced germinability of conidia 96, 80, 73, and 58 per cent, respectively. One per cent sodium tetrachloro-p-benzoquinone dissolved in a 0.32 per cent sodium hydroxide solution reduced germinability 47 per cent, whereas 1 per cent 4-chloro-1, 2-benzoquinone dioxime dissolved in 1 per cent ammonium hydroxide reduced germinability only 39 per cent. None of these materials, however, prevented the production of new conidia. Apparently, therefore, none penetrated the twigs sufficiently to reach and kill the mycelium therein.

In early February, 1942, before resumption of tree growth but after sporodochia became abundant on hold-over twigs, the following materials were applied in one apricot and two almond orchards: 0.5, 0.3, and 0.15 per cent solutions of sodium dinitro-o-cresylate; 0.15 per cent emulsion of dinitro-o-cresol; 0.37 and 0.5 per cent solutions of sodium orthophenyl phenate, sodium tetrachloro-phenate, and sodium pentachloro-phenate; 0.15 per cent of orthophenyl-, tetrachloro-, and pentachloro-phenols, which were dissolved in petroleum oil; and 0.5 per cent tetrachloro-p-benzoquinone dissolved in a 0.3 per cent aqueous solution of sodium hydroxide. About 2 to 3 weeks after spraying, when the trees began to bloom, the conidia in sprayed and nonsprayed trees were tested for germinability.

According to the results from these tests the highest reductions in germinability were 53 and 55 per cent, given by 0.5 per cent sodium dinitro-o-cresylate and 0.37 per cent sodium tetrachloro-phenate, respectively; the next highest was 44 per cent given by pentachloro-phenol in oil. This low reduction in the abundance of viable inoculum was reflected in one orchard where the disease developed. In this orchard the 53 per cent reduction in viable conidia by sodium dinito-o-cresylate was followed by only a 12 per cent reduction of blossom blighting.

As the eradicant effects of the materials in field tests were much less than their effects in the lath house tests, an effort was made to determine the reason. One reason seemed to be the failure of the materials to penetrate into the mass of conidia as they occur on the sporodochia. In two of the field trials a wetting agent was added. This material previously had been shown to increase the penetration of conidial masses by dinitro-o-cresylates and chloro-phenates. Despite the addition of this material, however, the spores at the center of the sporodochia were unaffected by the sprays, though the spores at the periphery were largely killed.

Possibly another reason for the inconsistency between lath-house and field results is the high solubility of the sodium salts of dinitro-o-cresylate, and the chloro-phenate. If, as happened in some of the field tests, heavy rains occur a few days after the sprays are applied, the materials may be washed from the tree before they exert their maximum influence against the fungus. Where twigs sprayed with sodium dinitro-o-cresylate were kept moist but were protected from rains by a bell jar, the percentage of spores killed within 5 days was ordinarily much higher than where twigs were exposed to rains.

## EFFECT OF SODIUM DINITRO-O-CRESYLATE, SODIUM ORTHOPHENYL-PHENATE, AND THE SODIUM CHLORO-PHENATES ON CONIDIA AND MYCELIUM OF CORYNEUM BEIJERINCKII

Calcium arsenite applied in January was found to reduce the incidence of the infection of almond leaves by *Coryneum beijerinckii* in the spring. In most cases the reduction was noticeable only where the trees had been sprayed with 4 pounds of calcium arsenite to 100 gallons of water. In one instance this concentration killed 75 per cent of the conidia of *C. beijerinckii* within blighted apricot buds. Concentration of 2 and 3 pounds to 100 gallons of water, on the other hand, failed to satisfactorily reduce apricot fruit infection by this fungus. As a concentration of 4–100 is too injurious to use on apricot trees, which are even more tolerant of arsenites than almonds, calcium arsenite apparently is not a satisfactory eradicant against this fungus.

In order to determine if the benzene derivatives were effective against *Coryneum beijerinckii,* preliminary tests were carried out in the lath house. Twigs of apricot and peach, bearing dormant buds blighted by the fungus, were collected in the winter. These were stood upright in moist sand and sprayed with various concentrations of the test materials. After drying the twigs were held in the lath house for one week. In all tests rains fell during this period.

According to the data in figure 3 all materials were noticeably lethal to the conidia of *Coryneum beijerinckii* borne on the inside of the diseased dormant buds. The most effective were sodium dinitro-o-cresylate, triethanolamine dinitro-o-cresylate, sodium pentachloro-phenate, and sodium tetrachloro-phenate. Somewhat less effective were 4-chloro-1, 2-benzoquinone, dioxime, tetrachloro-p-benzoquinone, and sodium trichloro-phenate. The least effective was sodium orthophenyl-phenate. Most of the materials appeared to be equally effective at 0.5 and 1 per cent, but all were less effective at 0.25 per cent.

In other tests, twigs sprayed with sodium dinitro-o-cresylate and the chloro-phenates were kept in moist chambers to prevent the sprays from drying. Within 48 hours most of the conidia inside the diseased buds were badly shriveled.

Sodium dinitro-o-cresylate, 0.5 and 0.3 per cent, applied to almond trees on January 29 did not materially reduce the disease subsequently developing on fruit and leaves, although an examination at the beginning of bloom showed that but few viable condia were present in blighted buds and on the surface of blighted spurs. Judging from the results given in table 2 and discussed below, it seems likely that between the time this examination was made and the development of leaves and fruit the fungus might have produced a new lot of conidia in buds on the treated trees.

The effect of sodium tetrachloro-phenate, sodium pentachloro-phenate, and tetrachloro-p-benzoquinone on conidia in apricot buds is shown in table

2. The sprays were applied on February 11 as the blossom buds were swelling. Examinations at the beginning (Feb. 18) and at the end of bloom (March 3) showed few viable conidia in buds sprayed with the chlorophenates, but somewhat more in those sprayed with the benzoquinone. On May 14, however, after the disease had made its appearance on fruit there

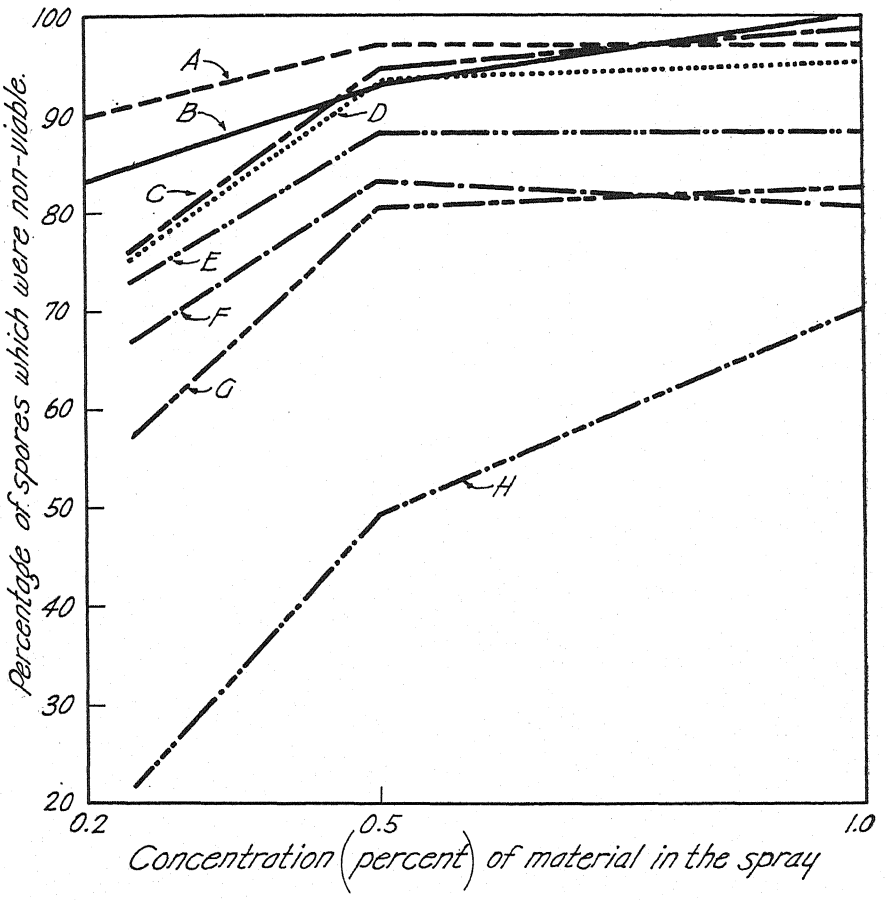



FIG. 3. Effect of spraying with different materials on the conidia of *Coryneum beijerinckii* in the diseased peach buds. A. Sodium dinitro-o-cresylate. B. Triethanolamine dinitro-o-cresylate. C. Sodium pentachloro-phenate. D. Sodium tetrachloro-phenate. E. 4-chloro-1, 2-benzoquinone dioxime dissolved in ammonium hydroxide. F. Sodium trichloro-phenate. G. Tetrachloro-p-benzoquinone dissolved in sodium hydroxide. H. Sodium orthophenyl-phenate.

were numerous viable conidia in buds sprayed with all materials. Leaf infection was too sparse for test purposes, but fruit infection was somewhat more abundant. The data (Table 2, column 5) show that none of the materials significantly reduced the disease on the fruit.

## DISCUSSION

Since Keitt[5] described the eradicant effect of sodium dinitro-o-cresylate (Elgetol) against the perithecial stage of *Venturia inaequalis* (Cke.) Wint. in over-wintered apple leaves, a number of apparently successful attempts to control this and other diseases have been reported. So far as the present writer is aware, however, no tests of other cresylates, of the phenates, or of related compounds have been reported. Although, on the whole, unsuccessful as eradicants against the fungi tested herein, the phenates, particularly tetrachloro- and pentachloro-phenate, show enough promise to be tried against such fungi as have yielded to sodium dinitro-o-cresylate.

TABLE 2.—*Effect of spraying with the chloro-phenates and tetrachloro-p-benzoquinone on the conidia of Coryneum beijerinckii in diseased buds and on apricot fruit infection, 1942*

| Treatment on February 11[a] | Percentage spores nonviable when taken from buds on | | | Percentage fruit infected on May 14 |
|---|---|---|---|---|
| | Feb. 18[b] | March 3[c] | May 14[d] | |
| Nonsprayed | 15 | 21 | 27 | 17 |
| Sodium tetrachloro-phenate, 0.5 per cent | 84 | 97 | 39 | 12 |
| Sodium pentachloro-phenate, 0.5 per cent | 90 | 96 | 33 | 14 |
| Tetrachloro-p-benzoquinone, 0.5 per cent[e] | 57 | 89 | 21 | 18 |
| Difference for significance at 99:1 odds | 11 | 5 | 9 | 6 |

[a] An organic wetting agent, 0.1 per cent added to each spray.
[b] Blossoms opening.
[c] Fruit beginning to grow.
[d] Fruit ⅓ grown.
[e] To dissolve this material 5 oz. of sodium hydroxide was added for each pound.

None of these materials approached the effectiveness of calcium arsenite in suppressing the development of sporodochia by *Sclerotinia laxa*. Partial suppression is little better than no suppression, since the fungus produces such an abundance of sporodochia, especially in almond trees. The relation of the degree of sporodochial suppression to the degree of reduction in blossom infection is illustrated in table 1, where a 95 per cent reduction of sporodochial development in 1939 was accompanied by a 78 per cent decrease in blossom blighting, whereas an 81 per cent reduction in the same year was accompanied by only a 12 per cent decrease.

One weakness of these materials as eradicants against both *Sclerotinia laxa* and *Coryneum beijerinckii* is their failure to kill the mycelium within the twigs and buds. A second point of weakness is their failure to penetrate the masses of conidia produced by *Sclerotinia laxa* on the sporodochia. It may be that their penetration into the sporodochia will be increased by a

[5] Keitt, G. W. Toxicity of the sodium salt of dinitro-o-cresol to *Venturia inaequalis*. Science (n. s.) **90**: 139–140. 1939.

proper supplementary material added to the spray, but such a material is yet to be found. A third point of weakness is believed to be their solubility. How much can be done with less soluble salts is yet to be shown.

To a certain extent failure of sodium dinitro-o-cresylate to lower the incidence of the shot hole on almond leaves and fruit is attributable, possibly, to occurrence of secondary infection. With this disease the critical period for attack of leaves and fruit extends from the time they appear until spring rains of sufficient length to allow for infection are at an end. The fungus produces conidia in abundance on infected blossoms and very young leaves, hence each one is a source of conidia for secondary infection.

## SUMMARY AND CONCLUSIONS

Of 41 materials tested for ability to eradicate the holdover stage of *Sclerotinia laxa* from apricot and almond trees, only 6 showed enough promise for further tests. These were 0.5 and 0.3 per cent solutions of sodium dinitro-o-cresylate (Elgetol), and 1 per cent solutions of sodium tetrachloro-phenate, sodium pentachloro-phenate, sodium orthophenyl-phenate, tetrachloro-p-benzoquinone, and 4-chloro-1, 2-benzoquinone dioxime.

In extended trials these materials showed only moderate ability to prevent the sporodochia (the conidia-bearing mats) from developing on the blighted twigs during the winter primarily because they did not kill the mycelium of the fungus within these twigs.

After sporodochia developed, however, the materials under some conditions were fairly effective in destroying them and the conidia borne thereon. Sodium dinitro-o-cresylate, sodium tetrachloro-phenate, and sodium pentachloro-phenate proved somewhat more injurious to the sporodochial stage than sodium orthophenyl-phenate, tetrachloro-p-benzoquinone or 4-chloro-1, 2-benzoquinone dioxime. Wide variability in the percentage of conidia killed, however, was experienced in different tests. In orchard tests in 1942 none reduced the primary inoculum sufficiently to insure an appreciable lowering of the amount of blossom infection by the fungus.

Triethanolamine dinitro-o-cresylate, ammonium dinitro-o-cresylate, dinitro-o-cresol, orthophenyl-phenol, tetrachloro-phenol, and pentachlorophenol, at the concentrations used, proved no more effective than the sodium salts.

The sodium salts of dinitro-o-cresol and the two chloro-phenols were about equally effective in killing the conidia of *Coryneum beijerinckii,* which are produced within blighted dormant buds on apricot, peach, and almond and constitute the primary inoculum for fruit and leaf infection in the spring. Within one week after applying 0.5 or 1 per cent solutions of these materials to diseased twigs, 80 to 95 per cent of the conidia were dead. Orchard tests, however, proved that the fungus later produced numerous new condia within sprayed buds, apparently because the spray had not killed the mycelium within the host tissue. Unsatisfactory control of fruit

and leaf infection in almond and apricot resulted, both because of this production of new conidia and possibly because of secondary infection arising from conidia produced on leaves and blossoms that were infected by primary inoculum from the buds.

Unless these materials are rendered more capable of penetrating the conidial masses on the sporodochia of *Sclerotinia laxa* and of entering the tissues and killing the mycelium both of *S. laxa* and *Coryneum beijerinckii* in the diseased twigs, they apparently will not be successful as eradicant sprays against the diseases in question.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA.

# EFFECTS OF EXTRACT OF WESTERN RED-CEDAR HEARTWOOD ON CERTAIN WOOD-DECAYING FUNGI IN CULTURE[1]

CHESTER M. SOUTHAM[2] AND JOHN EHRLICH[3]

(Accepted for publication September 7, 1942)

## INTRODUCTION

Previous workers have shown that the water-soluble extractives of western red-cedar (*Thuja plicata* D. Don) heartwood include a substance, probably phenolic, which is toxic to *Lenzites saepiaria* Wulf. ex Fr., *Lentinus lepideus* Fr., *Fomes annosus* (Fr.) Cooke, and "Madison 517"[4] (4, 7, 1, 2). Removal of this substance by water leaching results in a marked reduction in the well-known resistance of the heartwood to fungal decay (4, 7, 5, 6). In spite of the natural durability of western red-cedar heartwood in service, heart rot is common in mature stands. Since fungi evidently invade the heartwood in spite of the toxic substance, it seemed desirable to investigate the effects of various concentrations of western red-cedar heartwood extract on growth of certain common wood-decaying fungi in culture.

## EXPERIMENTAL WORK

### Preparation of Extract and Media

The extract used was prepared by reducing to sawdust the heartwood of a bolt cut from the lower part of the trunk of a freshly felled western red-cedar approximately 15 inches in diameter at breast height (4.5 feet). A 950-g. portion of this sawdust was placed in flasks and covered with distilled water. The mixture was kept, with occasional shaking, for a period of two weeks, during which time it was maintained at a temperature of 100° C. (by means of flowing steam) for a total of 50 hours. No attempt was made to avoid oxidation of the extractives. Upon filtering through a Buchner funnel, 8 l. of filtrate were obtained. Since 8000 ml. of filtrate were recovered from 950 g. of wood, 8.4 ml. contained the extract from 1 g. of wood. A 1-per cent solution (defined as a solution containing the extract from 1 g. of wood in 100 ml. of solution) was prepared by taking 8.4 ml. of the above extract and making up to 100 ml. with distilled water. This system was employed in order to provide a standard method of designating concentrations of extracts based on the weight of wood represented per unit volume of extract. When the results of the present experiments are compared with those of a preliminary experiment, in which an-

[1] Harry S. Owens, formerly Assistant Professor of Chemistry, University of Idaho, kindly criticized the manuscript.

[2] University Teaching Fellow, University of Idaho.

[3] Associate Professor of Forestry, University of Idaho.

[4] "Madison 517" is culture number 517 of the Forest Products Laboratory, Madison, Wisconsin. This culture is the same one used by Anderson and Sherrard (1), who referred to it as *Fomes annosus* (personal communication from E. C. Sherrard). Cartwright (in Trans. Brit. Myc. Soc. 22: 232–233. 1939) has shown that it is probably *Polyporus tulipiferus*.

other batch of extract was used, and with those of other workers (7, 2) after converting their figures into terms of percentage, it becomes evident that such percentage figures denote rather definite degrees of toxicity, although it is realized that the degree of toxicity for a given concentration of extract will vary with the concentration of toxic substances in the wood sample and with the completeness of the extraction.

Media containing the toxic extract were made according to the following formula:

2 g. "Difco Bacto-agar"
2.5 g. desiccated "Difco malt extract"
Volume of aqueous heartwood extract necessary to furnish desired percentage
Volume of water to make up to 100 ml.

In order to prepare media containing over 10 per cent of extract it was necessary to concentrate the extract. For this purpose some of the extract was evaporated so that 4 ml. represented 1 g. of wood. The evaporation was carried on at 50° C., at atmospheric pressure.

## Manipulation of Fungi

The following fungi were used in the tests: *Coniophora puteana* (Schum. ex Fr.) Karst. = *C. cerebella* (Pers. ex Fr.) Schroet., *Fomes officinalis* (Vill. ex. Fr.) Faull = *F. laricis* (Jacq. ex Fr.) Murr., *F. pinicola* (Swartz) Cooke, *F. roseus* (Alb. and Schw. ex Fr.) Cooke, *Lentinus lepideus* Fr., "Madison 517," *Polyporus schweinitzii* Fr., *P. sulphureus* Bull. ex Fr., *Poria xantha* (Fr.) Lind. forma *crassa* (Karst.) Baxt., *Trametes serialis* Fr., and *T. subrosea* Weir.

Circular planting pieces 1 sq. cm. in area were transferred, mycelium side up, from the margins of actively growing malt-agar, Petri-dish cultures to the centers of duplicate Petri dishes of test media containing various concentrations of western red-cedar heartwood extract. Duplicate control plantings were made on medium prepared as described above but without any extract. The diameter of the resulting mycelial mats was measured every 4 days until the mycelium on the control medium reached the edges of the plates. Growth was recorded as percentage of normal growth (diameter of fungus on test medium × 100 / diameter of fungus on control medium).

## Results of Growth Tests

Since measurements of duplicate cultures were found to be practically identical (with a few exceptions on media containing high concentrations of extract), only the means of measurements in duplicate plates are presented. Table 1 shows that any considerable concentration of the extract (4 per cent or over) retarded the rate of growth of all of the tested fungi, and that somewhat higher concentrations completely prevented growth. This is in agreement with the findings of other workers (4, 7, 1, 2, 3).

TABLE 1.—*Percentage of normal growth on malt agar containing various concentrations of western red-cedar heartwood extract*

| Species tested[a] | Age of culture | Percentage concentration of extract in medium[b] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ¼ | ½ | 1 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 |
| | *Days* | *Per cent of normal growth*[c] | | | | | | | | | | | | | | |
| *Trametes serialis* | 4 | 100 | 100 | 90 | 85 | 60 | 40 | 5 | G | G | G | G | 0 | 0 | 0 | 0 |
| | 8 | 100 | 95 | 95 | 82 | 60 | 45 | 35 | 35 | 40 | 35 | 30 | G | F | 0 | D |
| *Fomes roseus* | 4 | 100 | 110 | 110 | 110 | 92 | 60 | G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 8 | 100 | 105 | 108 | 95 | 90 | 70 | 53 | 50 | 40 | 37 | 0 | 0 | 0 | 0 | 0 |
| | 12 | 100 | 100 | 100 | 95 | 90 | 80 | 65 | 63 | 60 | 50 | F | F | F | 0 | D |
| *Trametes subrosea* | 4 | 110 | 100 | 95 | 90 | 60 | G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 8 | 107 | 105 | 100 | 100 | 80 | 60 | 40 | 30 | 30 | 33 | 0 | 0 | 0 | 0 | 0 |
| | 12 | 105 | 100 | 100 | 100 | 90 | 83 | 60 | 45 | 37 | 40 | 0 | 0 | 0 | 0 | D |
| "Madison 517" | 4 | 100 | 100 | 100 | 90 | 75 | 60 | 45 | 25 | 28 | 24 | 0 | 0 | 0 | 0 | 0 |
| | 8 | 100 | 95 | 90 | 90 | 85 | 73 | 60 | 30 | 30 | 25 | F | F | F | 0 | D |
| *Lentinus lepideus* | 4 | 100 | 100 | 100 | 100 | 80 | 60 | G | G | G | 0 | 0 | 0 | 0 | 0 | 0 |
| | 8 | 100 | 105 | 110 | 95 | 83 | 70 | 50 | 45 | 30 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 12 | 110 | 100 | 100 | 90 | 80 | 75 | 60 | 55 | 35 | F | F | F | F | 0 | D |
| *Polyporus sulphureus* | 4 | 100 | 105 | 105 | 100 | 55 | G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 8 | 100 | 105 | 105 | 100 | 80 | 63 | 40 | 32 | 32 | D | D | D | D | D | D |
| *Fomes officinalis* | 4 | H | H | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 8 | 150 | 177 | G | G | G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 12 | 125 | 134 | 65 | G | G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 16 | 120 | 125 | 81 | 71 | 64 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 20 | 115 | 113 | 90 | 80 | 80 | 40 | G | F | F | F | F | F | F | F | F[d] |
| *Polyporus schweinitzii* | 4 | 100 | 105 | 100 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 8 | 100 | 100 | 100 | 70 | F | F | F | F | F | D | D | D | D | D | D |
| *Fomes pinicola* | 4 | 100 | 90 | 80 | 60 | G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 8 | 95 | 85 | 77 | 62 | 42 | G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 12 | 100 | 85 | 78 | 70 | 48 | 20 | D | D | D | D | D | D | D | D | D |
| *Coniophora puteana* | 4 | 100 | 100 | 100 | 83 | G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 8 | 100 | 100 | 100 | 90 | 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 12 | 100 | 100 | 100 | 95 | 58 | D | D | D | D[e] | D | D | D | D | D | D |
| *Poria xantha* f. *crassa* | 4 | 105 | 120 | 95 | G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 8 | 130 | 130 | 110 | 10 | G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 12 | 110 | 110 | 105 | 88 | 55 | D | D | D | D | D | D | D | D | D | D |

G = growth evident on planting piece but not on test medium.
H = hormesis: stimulation indicated by growth on test medium prior to growth on control medium.
F = fungistasis: growth prevented but mycelium not killed.
D = death: mycelium failed to grow when planting piece transferred to normal medium.

[a] Fungi arranged in order of decreasing growth.
[b] Grams of wood represented per 100 ml. of medium.
[c] "Normal growth" refers to growth on control medium.
[d] Another batch of extract was found to be lethal to *Fomes officinalis*. Growth rates of other fungi on various dilutions of this extract indicate that its concentration was probably between 30 and 35 per cent.
[e] In another series of tests one planting piece of *Coniophora puteana* grew on test medium containing 12 per cent of extract.

In order to learn whether the planting pieces that did not grow were dead, or merely static, they were removed to ordinary malt-agar medium after the test period. This test showed that all non-growing planting pieces of *Coniophora puteana, Fomes pinicola, Polyporus sulphureus, Poria xantha* f. *crassa,* and *Trametes subrosea* were dead, whereas some planting pieces of *Fomes officinalis, F. roseus, Lentinus lepideus,* "Madison 517," and *Poly-*

*porus schweinitzii* resumed growth. These results indicate that within the test period some concentrations of the toxic extract were completely fungistatic, but not lethal, to certain of the fungi.[5] This study did not indicate whether continued exposure would kill the static plantings, or whether they would eventually grow.

An increase in growth rate of many of the fungi in extreme dilutions of the extract is also evident from the data in table 1. This accelerated growth rate tended to decrease with time and approach the normal rate (*e.g., Fomes officinalis*). It is possible that this accelerated growth is caused by some nutritive portion of the extract, but such an explanation would hardly account for the subsequent slowing down (towards the normal growth rate) because, if this explanation were correct, the increased rate of growth would presumably be maintained as long as there remained an excess of medium to furnish nutrients. It is more likely that the phenomenon represents an initial response, followed by progressive desensitization to subinhibitory concentrations of a toxic constituent of the extract. The term *hormesis* (adj. *hormetic*) is proposed to designate such a stimulatory effect of subinhibitory concentrations of any toxic substance on any organism.

## Acquired Resistance

Table 1 also shows that fungistasis decreased with time (*e.g., Fomes officinalis*) as if the fungi, after continued association with the toxic material, became able to tolerate or resist it, with a consequent increase in growth rate. This decrease in fungistasis suggested that a fungus might become adapted to high concentrations of toxic extract if it were gradually subjected to successively higher concentrations. In order to test this possibility plantings from the highest percentage of extract (in malt-agar medium) on which each fungus originally grew were transferred to a

TABLE 2.—*Concentrations of extract allowing growth before and after adaptation*

| Species tested | Original tests | | After adaptation |
|---|---|---|---|
| | Highest concentration on which growth occurred | Lowest lethal concentration | Highest concentration tested on which growth occurred |
| | *Percentage concentration of extract in medium* | | |
| *Trametes serialis* | 18 | 22–24[a] | 24 |
| *Fomes roseus* | 14 | 22–24 | 24 |
| *Trametes subrosea* | 14 | 16–24 | 18 |
| "Madison 517" | 14 | 22–24 | 24 |
| *Lentinus lepideus* | 12 | 22–24 | 22 |
| *Polyporus sulphureus* | 12 | 14 | 24 |
| *Polyporus schweinitzii* | 6 | 14 | 14 |
| *Fomes pinicola* | 6 | 8 | 18 |
| *Coniophora puteana* | 4 | 6 | 14 |
| *Poria xantha* f. *crassa* | 4 | 6 | 14 |

[a] The precise lethal concentrations were not determined for the first five species.

[5] "Toxic" is used in the sense of injurious, whether fatal or not; "lethal," in the sense of fatal; and "fungistatic," in the sense of inhibitory but not lethal.

slightly higher percentage of extract. It was found that each of the fungi had become able to grow on these higher concentrations. The fungi were then re-transferred to still higher concentrations of extract. By this procedure it was found that each of the tested fungi could be made to grow on concentrations of extract well above the highest concentration on which each originally grew.[6] The pertinent data from these tests are recorded in table 2. The fungi undoubtedly could have been made to grow on much higher concentrations of the toxic extract by this method of adaptation.

### Zone of Decoloration on Test Media

In media containing extract a zone of decoloration was observed extending a centimeter or less beyond the periphery of the mycelium of *Fomes officinalis, Fomes pinicola, Poria xantha* f. *crassa,* and *Trametes serialis* (Fig. 1, A, B). In plantings of *Poria xantha* f. *crassa* the zone of decoloration was present even around killed planting pieces (Fig. 1, C).

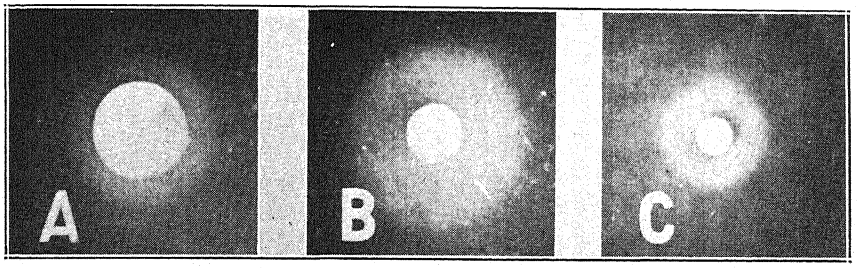



FIG. 1. Zone of decoloration in medium containing western red-cedar heartwood extract. A, decoloration around growing culture of *Fomes pinicola;* B, decoloration around growing culture of *Poria xantha* f. *crassa;* C, decoloration around killed planting piece of *P. xantha* f. *crassa.*

The possibility that this loss of color was the result of diffusion from the extract medium toward the planting pieces seems untenable for several reasons. Decoloration did not occur with all fungi, and was observed with killed planting pieces of only one fungus. Observations indicate that the color is held in an adsorption complex on the agar,[7] in which state diffusion would be impossible. The margin of the decolorized zone was quite definite, whereas if the loss of color were the result of simple diffusion into the planting piece a gradual transition would be expected.

The zone of decoloration, therefore, is considered to have resulted from leaching of metabolic products from the fungus into the surrounding

[6] *Fomes officinalis* was not included in these tests.

[7] Two facts indicate that the brown color of the extract is tied up in an adsorption complex when mixed with the medium: no Liesegang rings were present in the decolorized zone, which might be expected if the chromophore were capable of diffusion; the zone of decoloration was permanent, still being evident around the killed plantings of *Poria xantha* f. *crassa* two months after planting, whereas if diffusion of color were possible the decolorized zone would eventually darken. Apparently the chromophore is not colloidal when in water solution, because after filtering the extract through a Berkefeld filter the filtrate gave no indication of colloidality but still retained its brown color. It seems likely, therefore, that the color is held by adsorption to the particles of agar when in the medium.

medium where a chemical reaction produced the lighter color. The killed planting pieces of *Poria xantha* f. *crassa* must have produced enough of these metabolic substances before death to cause decoloration. Since this phenomenon occurred on media containing extract, and not on the control medium, it must have been the extract, and not the malt, that lost color.

Little is known concerning the chemistry of the cedar extract. Anderson and Sherrard (1) obtained two fractions from the aqueous extract, one of which they purified and identified as dehydroperillic acid. This substance was colorless and non-toxic to "Madison 517" in culture. They found the other fraction colorless also, toxic to "Madison 517," and probably phenolic.[8] Since phenolic compounds, although commonly colorless, characteristically oxidize to give a brownish color, it would seem to follow that the toxic portion of the extract becomes brownish on exposure to air.[9] If this be so, it is highly probable that the toxic portion of the extract is involved in the decoloration reaction. This would mean that the fungi causing the zone of decoloration do not grow in the presence of the toxic portion of the extract, but change it chemically (apparently to a non-toxic material) before advancing. This theory is strictly applicable only to those fungi in which the zone of decoloration was observed. There is no evidence at the present time to indicate the possible mechanism of growth of other fungi when subjected to cedar extract; yet it is quite possible that a similar mechanism holds for other fungi, the products of the reaction between the extract and the fungal product not being distinguishable, on the basis of color, from the unchanged extract.

Ability to overcome increasingly high concentrations of the toxic heartwood extract by virtue of this mechanism may well explain the advance of decay-producing fungi through the heartwood of living western red-cedar trees.

### Other Cultural Characteristics on Extract Media

In young cultures (2–3 cm. in diameter) of "Madison 517" the extract medium beneath the planting piece was darkened to an orange-brown. This color advanced as the mycelium grew, the area beneath the planting piece fading to a straw color. On old cultures, which completely filled the plates, the orange-brown color eventually disappeared completely, leaving the medium straw-colored throughout. Since these color changes were more pronounced on higher concentrations of extract, and since the ring of

[8] The present study has also furnished evidence that the extract contains a phenolic group: (a) a very dark orange color is produced when a few drops of ferric chloride are added to the extract; (b) a white precipitate is formed with bromine water; (c) the extract, which is straw-colored when fresh, gradually darkers to dark brown upon exposure to air. (Growth tests with old, darkened extract gave the same results as tests with corresponding concentrations of fresh, light-colored extract, indicating that oxidation does not alter degree of toxicity, a characteristic that also holds for phenol.) Reactions a and b also occur after all colloidal particles have been removed by filtration through a Berkefeld filter.

[9] Possibly some non-toxic portion of the extract is also oxidized by atmospheric oxygen to give a brown color, in which case the decoloration might not concern the toxic portion of the extract exclusively.

orange-brown advanced as the mycelium grew, it appears that the color resulted from changes in the extract caused by the fungus. This is another indication that this fungus causes chemical changes in the extract, and does not merely grow in its presence, although there is no evidence to indicate whether this color is a product of the toxic portion of the extract or of some non-toxic part.

Certain of the fungi (notably *Polyporus sulphureus*), which produce azonate mycelium on ordinary malt-agar medium, exhibited pronounced zonation on media containing high concentrations of extract.

Mycelial mats were almost invariably thicker on media containing extract than on ordinary medium.

## Incidental Data

When samples of the extract were allowed to evaporate to dryness, and weighed, 4.5 g. (average of 3 determinations) of extractives were obtained from each 100 g. of air-dry wood. This figure is probably low due to volatilization of solids during evaporation, because blocks (from another tree) when leached lost an average of 8.2 g. per 100 g. of oven-dry wood. Using this latter method Cartwright (2) obtained values of 5 per cent to 6.9 per cent.

The acidity of increasing concentrations of the extract was determined potentiometrically. It was found that the pH decreased rapidly to 3.5 for 8 per cent extract, beyond which there was very little change, a 24 per cent solution having a pH of 3.2. Such a curve is characteristic of the weak organic acids. Cartwright (2) reports that extracts from 2 trees showed pH values of 2.2 and 4.5. An attempt was made, with various cultures, to detect differences in pH between the decolorized zone and the unchanged medium beyond the decolorized zone. The results were so inconsistent that no conclusions could be reached.

High concentrations of extract prevented the agar from gelling completely, possibly due to its acidic reaction.

## SUMMARY AND CONCLUSIONS

The hot-water-soluble extractives of western red-cedar heartwood were removed at 100° C. from sawdust and mixed, in various concentrations, with malt-agar medium. Several species of wood-decaying fungi were planted on these media and their growth rates were measured. Growth characteristics of the fungi in the presence of the toxic extract were recorded. The following conclusions were reached.

Extreme dilutions of the extract are hormetic (stimulatory), at least to some fungi.

The degree of hormesis decreases with the age of the culture.

Any considerable concentration of the extract is fungistatic, and in sufficient concentration the extract is lethal to the tested fungi.

The degree of fungistasis decreases with the age of the culture.

Fungi can be adapted to normally lethal concentrations of the extract if they are grown on successively higher concentrations of the extract in a malt-agar medium.

Some, at least, of the tested fungi secrete metabolic products that decolorize the surrounding medium and apparently overcome its toxicity.

SCHOOL OF FORESTRY,
UNIVERSITY OF IDAHO,
MOSCOW, IDAHO.

LITERATURE CITED

1. ANDERSON, ARTHUR B., and E. C. SHERRARD. Dehydroperillic acid, an acid from western red cedar (*Thuja plicata*). Jour. Amer. Chem. Soc. 55: 3813–3819. 1933.
2. CARTWRIGHT, K. ST. G. The variability in resistance to decay of the heartwood of home-grown western red cedar (*Thuja plicata* D. Don.) and its relation to position in the log. Forestry, Jour. Soc. For. Gr. Brit. 15: 65–75. 1941.
3. PATTON, ROBERT F. Isolation of fungi from brown cubical heart rots of western red-cedar. University of Idaho, Master's thesis (unpublished). 1942.
4. SCHMITZ, HENRY. Preliminary note concerning the cause or causes of the durability of western red cedar, *Thuja plicata*. Idaho Forester 4: 46–47. 1922.
5. SELLERS, VICTOR O. A cultural study of the heart rots of western red-cedar in northern Idaho. University of Idaho, Master's thesis (unpublished). 1940.
6. SOUTHAM, CHESTER M. A study of the saprogenicity, and factors influencing decay, of certain brown-rot fungi on western red-cedar heartwood test blocks. University of Idaho, School of Forestry, Undergraduate thesis (unpublished). 1941.
7. SOWDER, A. M. Toxicity of water-soluble extractives and relative durability of water-treated wood flour of western red cedar. Ind. Eng. Chem. 21: 981–984. 1929.

## PHYTOPATHOLOGICAL NOTES

*The Bulb or Stem Nematode on Alfalfa, Sweet Clover, and White Clover.*—The bulb or stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, has a host range comprising over 300 plant species. Within this nematode species there exist biologic strains that infect only one host, and others that infect several hosts. The experiments recorded here show that a strain of *D. dipsaci* from alfalfa was capable of infecting 3 different hosts.

In April, 1942, a field of alfalfa, *Medicago sativa* L., near Reno, Nevada, was observed in which a high percentage of the plants was infected with *D.*

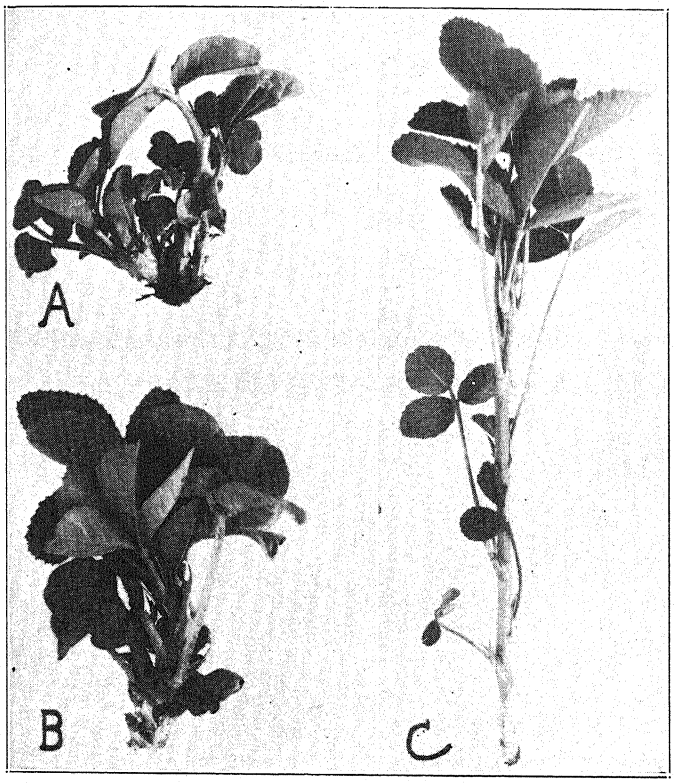



FIG. 1. Shoots of sweet clover plants. A and B. Naturally infected with *Ditylenchus dipsaci*. C. Not infected.

*dipsaci*. Many sweet clover plants, *Melilotus alba* Desr., were present in this field and at least 50 per cent bore swollen, distorted buds, heavily infected with stem nematode (Fig. 1). A few plants of white clover, *Trifolium repens* L., also were found with characteristic symptoms. Plants of red clover, *T. pratense* L., were present but apparently were not infected.

Under greenhouse conditions, cross inoculations were made between infected and noninfected alfalfa, sweet clover, and white-clover plants to de-

termine if the same strain of eelworm was parasitizing the 3 plant species found naturally infected in the field. Inoculations were made by scattering pieces of nematode-infected buds among the crowns of nematode-free plants and covering them with a thin layer of sand, kept moist to favor the nematodes. The following transfers were successful: alfalfa to alfalfa, alfalfa to sweet clover, sweet clover to alfalfa, sweet clover to sweet clover, and white clover to alfalfa. Check plants remained free from infection. Transfers were regarded as positive only when microscopic examination showed that the life cycle of the nematode was completed within the tissues of the host plant.

The experimental transfers, the natural plant infections in the field, and the fact that no apparent morphological differences exist among specimens from the three hosts, show quite definitely that this strain of *Ditylenchus dipsaci* was parasitic on alfalfa, sweet clover, and white clover.

Experiments under identical conditions, using alfalfa buds infected with *Ditylenchus dipsaci,* collected near Minden, Nevada, failed to produce infection on sweet clover. This may indicate that populations differing in host preference may exist in the two localities.—OLIVER F. SMITH, Associate Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, U. S. Dept. of Agriculture, Reno, Nevada, and MERLIN W. ALLEN, Assistant Nematologist, Division of Nematology, Bureau of Plant Industry, U. S. Dept. of Agriculture, Salt Lake City, Utah.

*Phoma terrestris in the Roots of Mature Maize Plants.*[1]—In the autumns of 1941 and 1942, *Phoma terrestris* Hansen was isolated alone, and in combination with *Fusarium moniliforme, Pythium* sp., or miscellaneous fungi, from pink roots of plants of yellow dent corn grown on the University farm, Madison, Wisconsin.

Pycnospores were formed in culture in 1941; in 1941 and 1942 the rose-color hyphae and olive-brown "pycnidial primordia" characteristic of *Phoma terrestris*[2] were visible in the root tissues. The slender pink hyphae had penetrated the stele (Fig. 1, A); the pycnidial primordia were found most often in the cortical layers of the root, within the cells of the endodermis (Fig. 1, B), and in the small rootlets (Fig. 1, C). This organism differed from the common run of fungi in that the rose color of the hyphae remained unchanged during the process of killing, dehydration, and embedding. Formol-acetic alcohol was the killing solution used, followed by alcohol, chloroform, and paraffin.

The fungus was isolated from the plants of several single crosses, among which there appeared to be some differences in susceptibility to the root injury.

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

[2] Hansen, H. N. Etiology of the pink-rot disease of onions. Phytopath. 19: 691–704. 1929.

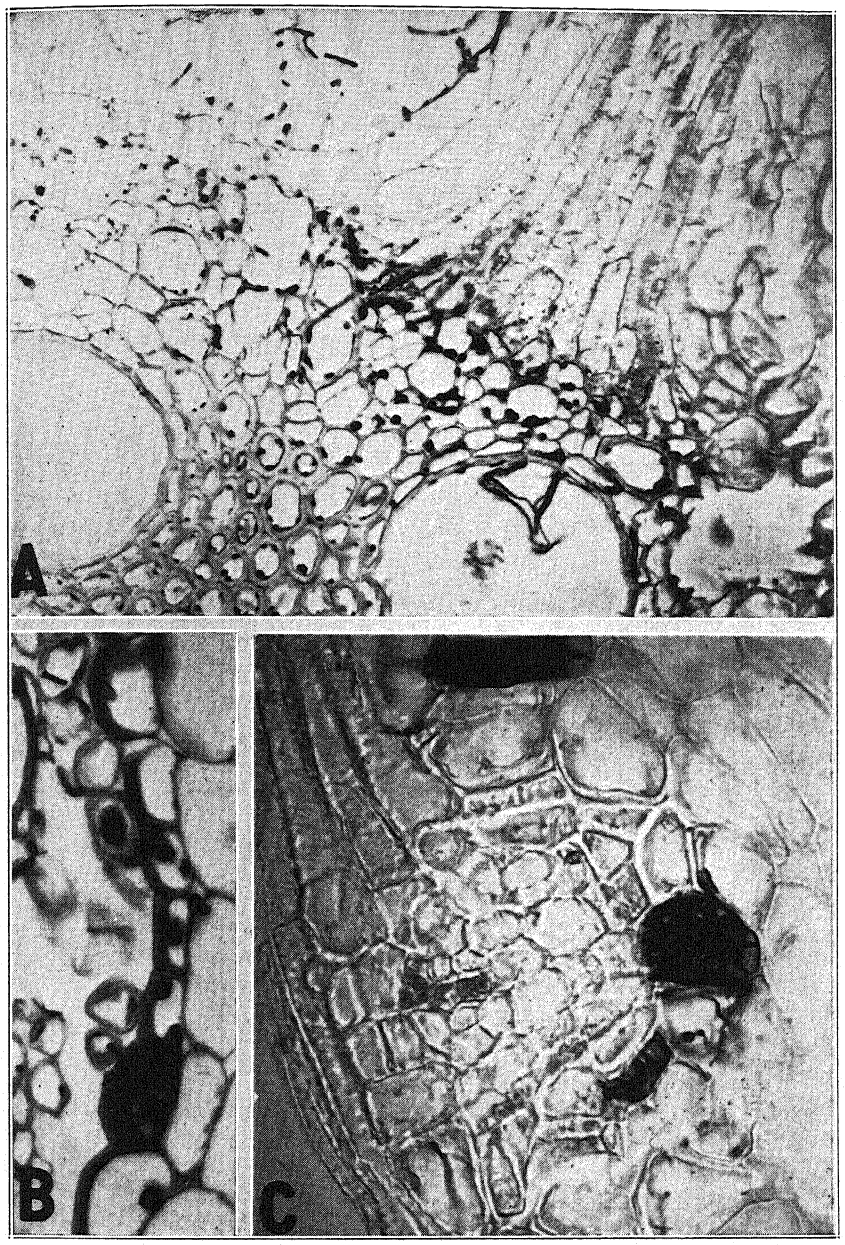



FIG. 1. Photomicrographs of paraffin sections of corn roots and rootlets showing *Phoma terrestris* within the tissues. A. Cross section of root showing the rose-color hyphae of the fungus in the cells and vessels of the stele. ×290. B. Cross section of root containing pycnidial primordium and olive-brown hyphae in the endodermal cells and rose-color hyphae in the stele. ×500. C. Pycnidial primordium in the cortex of a root, and both primordia and pink hyphae in the rootlet. ×425. All sections were lightly stained with fast green to render the host tissue visible. Safranin was used also in B, to differentiate the endodermal cells. The fungus needed no stain. (Photomicrographs made by Eugene H. Herrling, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.)

Kreutzer[3] included corn in his list of young crop plants slightly susceptible to attack by *Phoma terrestris*. His experiments were conducted with seedlings in flats of autoclaved soil. In the present case natural infection occurred in the field, and the plants were mature when examined. Consequently, it is not known at what stage in the development of the plants the fungus had entered the roots, nor the amount of injury caused by the attack. It is believed, however, that pink root of mature corn plants heretofore has not been ascribed to *P. terrestris* within the root tissue.—HELEN JOHANN, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

*Scolecospores in Diplodia macrospora.*[1]—While examining platings of rotted corn kernels from a market sample of the 1942 crop, from Wye Mills, Maryland, a culture of *Diplodia macrospora* Earle with abundant scolecospores recently was observed. This is of two-fold interest in that scolecospores have been hitherto unreported in *D. macrospora,* and, according to the records in the Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture, this is the first recorded incidence of this species of *Diplodia* in Maryland.

Whether or not the scolecospores occurred separately or in conjunction with the pycnospores within the same pycnidium was not ascertained, inasmuch as both spore forms were seen only after a mount had been made of a small cluster of crushed pycnidia scraped from the surface of the corn kernel. In her discovery of scolecospores in *D. zeae,* in 1939, Miss Johann[2] described this spore form as occurring either alone or with the bicellular pycnospores in the pycnidium.

The relative infrequency of *Diplodia macrospora* in Maryland is attested by the fact that during the course of a corn-ear-rot survey, now going into the 10th consecutive year, approximately 3,000 cultures of *D. zeae* were plated from this State before the first culture of *D. macrospora* was found. —PAUL E. HOPPE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, University of Wisconsin, Madison, Wis.

*Plot Technique for Disease-control Studies on Fine Turf.*—Investigation of chemical control of fine-turf diseases is conditioned by two major difficulties: 1. Paucity of experimental plots exhibiting the disease, and 2. Difficulty of finding sufficient area to minimize the error introduced by apportioning the small quantity of necessary chemicals.

Certain diseases, *e.g.,* dollar spot, are easily induced by artificial inoculation. The pathogen can be cultured readily on sterilized grain. If the

[3] Kreutzer, W. A. Host-parasite relationships in pink root of *Allium cepa.* II. The action of *Phoma terrestris* on *Allium cepa* and other hosts. Phytopath. 31: 907–915. 1941.

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, U. S. Department of Agriculture and the Wisconsin Agricultural Experiment Station.

[2] Johann, Helen. Scolecospores in *Diplodia zeae.* Phytopath. 29: 67–71. 1939.

inoculum be broadcast over the area, infection occurs in 2 to 3 days. Infection can be intensified by leaving the clippings of the first and second mowings after inoculation. Snowmold (*Typhula*) inoculum, sown broadcast, has given excellent results in 2 out of 3 years; yet, repeated attempts to induce large brown patch, Helminthosporium or Colletotrichum "melting-out" have failed. It has been necessary, therefore, to seek experimental areas on golf courses where these diseases occur naturally.

Golf-course greens vary from 3,500 to 7,000 square feet of turf. Generally of irregular shape, they present terrain of various levels and slopes. Many replications are needed to reduce the error of heterogeneity due to elevation, exposure, playing wear, and drainage. Areas of 1,000 square feet are de-

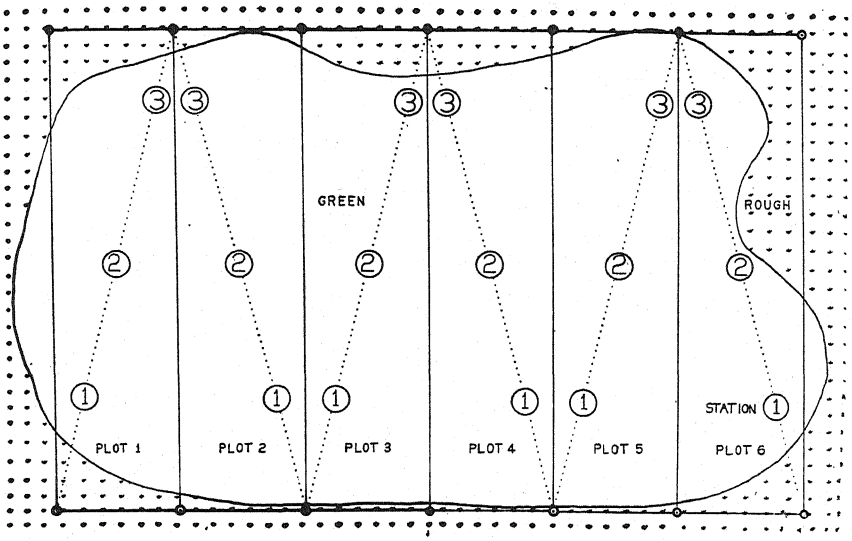



FIG. 1. Plot layout of 18th Green, West Course, Merion Golf Club, Ardmore, Penna.

sirable for treatment, since practical control measures are based on rates per 1,000 square feet. It would be almost impossible to lay out such plots on the average golf green without the aid of a surveyor. Moreover, treatments must be made intermittently throughout the growing season, a fact necessitating rapid relocation of the plots. These handicaps are easily overcome by the following technique:

Knowing the number of treatments to be used, estimate the area to be plotted, *e.g.*, 4 treatments—4,000 square feet; 6 treatments—6,000 square feet. Then measure long axis of green avoiding diagonals. Using measure of this long axis, calculate convenient width for rectangle required to give experimental area. With raised or cut-in greens, adjust width in direction of more accessible terrain. Locate corner stakes in rough (Fig. 1), drive to ground level to avoid interference with mower. Treat individual plot stakes in like manner. Whitewash each stake and small area surrounding it for subsequent easy relocation. Whitewash must be renewed once or twice because of growth and subsequent clipping of grass. On large and irregular greens residue areas often are big enough for replicate or additional treatments. On application of fungicide insert 6-inch spike behind each stake; stretch heavy twine to outline individual plots. Apply treatment uniformly over whole plot, rough and green alike.

For dollar spot, data may be satisfactorily taken by arbitrarily locating specific stations on a diagonal of each plot. If a 100-foot tape line be used to locate the diagonal, stations may be located at specific points. If infection is uniform the location of stations is a simple chore, but if scattered, one must be careful to insure representative areas. Three stations are used, each containing the area enclosed by a home-made wire hoop 3 feet in diameter. The spots per station are counted and averaged. Increase or decrease in average number of spots correlated strikingly with visual evidence of fungicide effectiveness.

For large brown patch and the "melting-out" complex, the station method was not so useful. A rating system had to be established employing the number of patches per foot of diagonal. Correlated with temperature and rainfall, this system worked satisfactorily. Data for each treatment were recorded weekly.—F. BEN STRUBLE, Graduate Stipend Scholar, Department of Botany, The Pennsylvania State College.

*A Method for Obtaining Single-spore Cultures of Agaricus campestris.*—To obtain single-spore cultures of *Agaricus campestris* it has heretofore been necessary to seed a mass of spores on a nutrient medium, and then isolate individual spores after they have germinated.[1,2] Although a high percentage of spores from such spore prints will often germinate, few single spores isolated from the same print will do so.[1] A method for germinating single spores would be useful because spores could be isolated with a micromanipulator and thus allow observation of germination and subsequent growth. Isolation and germination of pairs of spores from the same basidium would further facilitate genetic studies on this fungus.

It has been known for some time that spores of *Agaricus campestris* germinated best when in groups or in close proximity to growing mycelium of their species.[3] The assumption has been that a stimulatory substance may diffuse from the few germinating spores to others. These germinate and in turn stimulate others near them to germinate.

The writer attempted to use this stimulation in developing a method of inducing germination. A successful method is herewith described. A large number of uncontaminated spores were smeared on an agar slant with a sterile brush and allowed to germinate and grow for one to two weeks. After a solid mycelial mat had formed, the culture was killed by immersing the tube in boiling water until the medium was completely liquid. The dead mycelial mat was then removed aseptically and the sterile medium used for hanging drops on cover slips over Van Tieghem cells. From 30 to 50 per cent of the single spores of two commercial varieties of mushroom isolated and placed on this medium have germinated in replicated tests. Apparently the rate of germination is not so high for all varieties as one variety that resisted germination by mass seeding also germinated very poorly when single spores were isolated. A limited number of tests were made with medium staled by mycelial growth. A lower rate of germination was obtained here ranging between 20 and 30 per cent.

[1] Lambert, E. B. Principles and problems of mushroom culture. Bot. Rev. 4: 397–426. 1938.

[2] Lambert, E. B. A spore isolator combining some of the advantages of the LaRue and Keitt methods. Phytopath. 29: 212–214. 1939.

[3] Ferguson, M. A preliminary study of the germination of the spores of *Agaricus campestris* and other basidiomycetes. U. S. Dept. Agr., Bur. Pl. Ind. Bull. 16. 1902.

As checks against the experiments just described, 100 single spores were isolated and placed on unstaled medium and only about 5 per cent germinated. It would be difficult to obtain an adequate number of cultures in this way because of the large amount of work required. The new method should prove much more useful, as it insures a higher percentage of germination and thus makes it possible to get a more representative sample of the spore population with economy of time and effort.—D. J. DEZEEUW, Butler County Mushroom Farm Inc., West Winfield, Pa.

*Host-parasite Relationship in Rust-infected Oats.*—Initial cytochemical studies on the host-parasite relationship as presented by different varieties of oats (*Avena sativa*) infected by crown rust (*Puccinia coronata* Corda) were undertaken by the writers in 1942.

The varieties Bond, Bond × D69, Markton, Rainbow, Richland, and Victoria were inoculated with physiologic race 1 of *Puccinia coronata*. As soon as the rust sori had developed sufficiently for sectioning, freehand tangential sections of infected living leaf tissue were obtained from immediately beneath the sori and immersed in saline and sucrose solutions containing 1 per cent neutral red and stained *in vivo*. Like sections were immersed in a saturated solution of 2–6 dichloroquinone imide in a 2 per cent solution of sodium barbiturate buffered at pH 8.6. Still other sections were immersed in solutions of para-phenylenediamine, and molybdenum reagent, respectively.

Use of the above techniques afforded opportunity to note the comparative behavior of the phenolic compounds (pyridoxin, catechol, etc.), the polyphenol oxidases, and the distribution of the phosphorus compounds peculiar to the "resistant" or "susceptible" varieties of the host.

Response of the rust-infected host-plant cell may vary from sudden death of such cells to a progressive evolution of cell constituents making possible a prolonged survival of host cell and parasite. These extremes of response to infection, as well as all intermediate types of reaction, can now be interpreted cytochemically. A manuscript embodying the salient results of our investigation[1] of the problem is in preparation.—HARRY B. HUMPHREY and JEAN DUFRENOY, Bureau of Plant Industry Station, Beltsville, Md., and Louisiana State University, Baton Rouge, La.

[1] Grateful acknowledgment is here made to the members of the Department of Botany, Louisiana State University, for their collaboration and advice and for laboratory and greenhouse facilities placed at the disposal of the writers. The research here reported was conducted cooperatively by the Division of Cereal Crops and Diseases, Bureau of Plant Iudustry, Soils, and Agricultural Engineering, U. S. Department of Agriculture and the Louisiana State University.

# PARTICLE SIZE OF SULPHUR AND COPPER FUNGICIDES IN RELATION TO APPLE SCAB AND CEDAR-APPLE RUST CONTROL

J. M. HAMILTON, D. H. PALMITER, AND G. L. MACK[1]

(Accepted for publication October 22, 1942)

Within the past decade, the wettable sulphur fungicides, in both dry and paste forms, have steadily gained favor with fruit growers. They have in large measure supplanted lime-sulphur in commercial spray practice, because of the reduction in spray injury and increased yields of fruit.

Field tests with these proprietary wettable sulphur materials have demonstrated some to be superior to others in their effectiveness in controlling apple scab, *Venturia inaequalis* (Cke.) Wint. (9), and apple rust, *Gymnosporangium juniperi-virginianae* Schw. (10). During the course of these tests, the senior writer became convinced that the outstanding property of these products that could explain their differences in disease control was the size of the sulphur particles.

As early as 1913, Blodgett (2), working with sulphur dusts, pointed out that fine particles adhered to foliage more firmly than did coarser ones. Wilcoxon and McCallan (19), also, demonstrated in laboratory experiments on glass slides that fine-particle sulphur was inherently more toxic to germinating spores than an equal weight of coarse-particle sulphur. But the practical problem of correlating particle size to disease control in the orchard was complicated by the fact that all wettable sulphur fungicides necessarily contain adjuvants that markedly affect their resistance to weathering and often obscure the effects attributable to particle size. Further, the make-up of proprietary spray materials is subject to change from one season to the next.

In order to correlate particle size and disease control, it was necessary to eliminate the interfering effects of (1) varied and secret methods of manufacture, (2) the inclusion of different kinds and amounts of wetting agents, dispersing agents, and other ingredients. In 1937, large samples of a wettable sulphur and an insoluble copper fungicide were obtained whose compositions were known and were alike in all respects, except size of particles (11). The procurement of this material made exact testing possible.

The average particle sizes of these materials and of some of the more common commercial sulphur fungicides were determined and correlated with the results of disease control experiments obtained from their use in laboratory, greenhouse, and orchard.

[1] The writers are indebted to L. O. Weaver for assistance in conducting the greenhouse experiments and in the preparation of the manuscript.

Grateful acknowledgment also is due to R. C. Roark of the Insecticide Laboratory, U. S. Dept. Agr., Bureau of Entomology and Plant Quarantine, to the Stauffer Chemical Company, New York City, and the Micronizer Processing Company, Moorestown, New Jersey, for their cooperation.

Approved by the director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 531, November 7, 1942.

It is interesting to note that during the course of the studies reported herein, manufacturers have made concerted efforts to improve the effectiveness of commercial, powdered, fungicidal materials by grinding them finer and finer. In 1930, Streeter and Rankin (16) examined 6 representative commercial sulphur materials which varied between 12 and 30 microns for the diameter of the particle of average specific surface,[2] while in 1941, Gooden (6) reported that 42 samples varied between 5 and 25 microns for the same diameter. Values reported in this communication extend the lower limit of average particle size to 2 microns in commercial dry wettable sulphur fungicides. Thus, in 12 years, the lower limit of average particle size has been reduced sixfold, while the upper limit has remained practically stationary.

## METHODS OF MEASURING PARTICLE SIZE

The fineness of particles usually has been expressed as a function of the diameter, although several authors have pointed out the inadequacy of that expression when the individual particles are not all the same size and shape. Green (8) has shown that there can be no generalized expression for average particle size, and has discussed 6 different diameters that are equal only when every particle has the same size and shape. In a non-uniform powder, they vary as follows:[3]

$$d_4 > d_3 > d_2 \begin{matrix} < \\ = \\ > \end{matrix} D > \triangle > d_1$$

With a polydisperse material such as ground sulphur, a 4- or 5-fold variation between the smallest and largest of these diameters is not uncommon. It was, therefore, extremely important to choose that particular diameter best suited for denoting the particle size of fungicides.

The property of a powdered fungicide apparently most closely related to the degree of disease control obtainable was the specific surface area. Accordingly, the surface average diameter, $d_3$, was selected for designating the relative degree of fineness of fungicidal materials. The surface average diameter can be expressed mathematically as

$$d_3 = \frac{\sum nd^3}{\sum nd^2}$$

where $n$ is the total number of particles in the sample and $d$ is any diameter of the individual particle. More explicitly, the surface average diameter may be defined as the diameter of a sphere whose specific surface is the same as that of the sample. This idealized sphere need not exist in fact; *i.e.*, a sphere may be used to represent the average diameter of particles, none of which are spherical in shape. It will be seen that in any measurement of particle size, some assumption as to the general shape of the particles must be made.

[2] The writers have calculated these figures from the original data of Streeter and Rankin. The average specific surface is defined as the specific surface of the sample as a whole and not the arithmetic average of the specific surfaces of the individual particles.

[3] The symbols employed throughout this discussion are the same as those adopted by Green (8).

Two other of the diameters given by Green (8) have been used by previous workers to express the particle size of fungicidal materials. These are the *arithmetic mean diameter*, $d_1$, and the *diameter of the particle of mean weight*, $D$. They are calculated by means of the following equations:

$$d_1 = \frac{\Sigma nd}{\Sigma n}$$

$$D = \frac{\sqrt[3]{\Sigma nd^3}}{\Sigma n}$$

While it was not considered that these latter two diameters had the fundamental significance of the surface average diameter, they were included in a part of the particle-size measurements for the sake of comparing the different methods. No attempt was made to convert these different diameters to a common basis because of the uncertain value of the shape factor.

Four completely independent methods were employed for the determination of particle size. These were the sedimentation method (1), the air-permeation method (7), the direct microscopic method, and the microscopic-projection method (8, 13). The agreement between the results is remarkably good when it is considered that the properties which were actually measured were in order (1) a settling velocity, or rate of fall of particles through a liquid medium, (2) a permeability, or resistance to flow of air through a packed bed of particles, (3) the diameters of a number of particles, and (4) the number of particles of known weight that occupy a given area (Table 1). Lack of absolute agreement can be ascribed principally to differences inherent in the measurements and calculations. The possibility of changes in the samples themselves cannot be overlooked, since these experiments extended over a period of several years.

In general, it was considered that the *sedimentation method* using the Andreasen pipette gave the most reliable results (1). By this method, the distribution of particle sizes is obtained directly, so that the results may be subjected to critical analysis and checked by microscopic observation. In a few cases, agglomeration produced particles of irregular shape. These were assumed to settle as cubes according to the procedure of Andreasen, but otherwise, all particles were assumed to settle as spheres. This method has a most important advantage over other sedimentation methods in that any inert material, which also settles out, is not determined. But the presence of suspending agents, such as bentonite, can cause a lower reading by interfering with the normal sedimentation of the sulphur particles. Thus, the average particle size of Kolospray is probably somewhat larger than the apparent value of 7.7 microns (Table 2).

A second useful method was found to be the air permeation procedure as modified by Gooden and Smith (7). While the apparatus is somewhat complicated, the method is very rapid and well suited to routine work. The agreement between results obtained upon the same materials in two different laboratories is good (Table 1), and a further comparison of the sedimenta-

tion and air permeation methods shows remarkably concordant results (Table 2).

The air-permeation method can give low readings with materials which contain an insufficient number of very fine particles to completely fill the voids between the larger particles. The reason that the air permeation method is somewhat more accurate than the original liquid permeation method of Carman (3) is that the liquid acts as a lubricant, allowing the finer material to pack tightly into the voids between the larger particles. McMillen (15) has shown that a membrane consisting of a polydisperse powder has a smaller average pore diameter, when packed wet than when packed dry. Segregation of the finer particles is thus considerably reduced

TABLE 1.—*Comparison of results obtained from various methods of measuring particle size*

| Material | Year | Surface average diameter, $d_3$ | | | Number average diameter, $d_1$ | Weight average diameter, $D$ |
|---|---|---|---|---|---|---|
| | | Sedimentation | Air permeation | | Direct microscopic[a] | Microscopic projection |
| | | | A | B[b] | | |
| Special sulphur (fine) | 1937 | ..... | 3.5 | 4 | 2 | 7 |
| Micronized | 1940 | 3.6 | 3.7 | 5 | 3 | ..... |
| Special sulphur (medium) | 1937 | ..... | 6.6 | 7 | 6 | 10 |
| Special sulphur | 1937 | ..... | 10.3 | 12 | 18 | 13 |
| Mike | 1940 | 5.3 | 5.5 | 6 | 5 | ..... |
| Magnetic 70 (dry) | 1940 | 5.2 | 5.8 | 7 | 5 | ..... |
| Magnetic-Spray | 1940 | 8.3 | 9.7 | ..... | 8 | ..... |
| Sulcoloid | 1937 | ..... | ..... | 10 | 11 | 9 |
| Z–O[c] (fine) | 1938 | 1.6 | 1.4 | 1 | 6 | 1 |
| Z–O (medium) | 1938 | 10.0 | 8.2 | 6 | 10 | 7 |
| Z–O (coarse) | 1938 | 13.3 | 10.0 | 8 | 20 | 8 |

[a] The results listed in this column were obtained in the control laboratory of the Micronizer Processing Co., Moorestown, N. J.

[b] The results listed in this column were obtained in the insecticide laboratory of the U. S. Department of Agriculture, Bureau of Entomology and Plant Quarantine, under the direction of R. C. Roark.

[c] Z–O is a proprietary copper ammonium zeolite.

but not entirely eliminated by packing the powder dry. Uniform results are obtained by using the powder compactor designed by Gooden (5). This packing effect probably accounts for the low results obtained by the air permeation method for Microsulphur and Sulcoloid (Table 2), and for the medium and coarse samples of copper zeolite (Table 1).

Conversely, the air-permeation readings are too high for Magnetic-Spray and Kolospray (Tables 1 and 2). This is no criticism of the method, since these values are correct under the conditions of the measurements; but these materials contain aggregates, formed in the process of manufacture, which function as single particles in air and are dispersed into many particles in water. The fused bentonite sulphur material known as Kolofog is an ex-

treme example of this effect, since the average particle diameter in air is over 100 microns, while, in water, it is less than 1 micron. The air permeation method is, therefore, better suited to the determination of the particle size of dusts than of spray materials.

The microscopic measurement of particle size is considered by many to yield the most reliable results because it is visual and direct, and conforms more closely to the usual method of determining the size of larger objects. But, for irregular objects, the results are always too large, since the particles will tend to settle so that the two horizontal dimensions measured will be larger than the vertical dimension, which is not. This effect is apparent

TABLE 2.—*The particle size of some commercial wettable sulphur fungicides as determined by sedimentation and air-permeation methods*

| Brand name[a] | Surface average diameter | | Per cent by weight of particles less than 4 μ in diameter | Sulphur content | Manufacturer |
|---|---|---|---|---|---|
| | Sedimentation | Air permeation | | | |
| | *Microns* | *Microns* | | *Per cent* | |
| Koppers Dry Wettable | 2.0 | 2.2 | 89.6 | 91.0 | Koppers Products Company |
| Micronized Sulphur | 3.6 | 3.7 | 46.5 | 93.4 | Micronizer Processing Co. |
| Mike Sulphur | 5.3 | 5.5 | 21.2 | 92.5 | Dow Chemical Company |
| Sulforon | 6.6 | 6.6 | 11.4 | 98.0 | DuPont de Nemours & Co. |
| Micro-Spray | 6.7 | 5.9 | 9.7 | 92.5 | General Chemical Company |
| Kolospray | 7.7 | 11.2 | 8.2 | 83.5 | Niagara Sprayer & Chem. Co. |
| Magnetic-Spray | 8.3 | 9.7 | 7.4 | 98.5 | Stauffer Chemical Company |
| Sulfix | 8.8 | 8.8 | 7.0 | 97.3 | Sherwin-Williams Company |
| Microsulphur | 10.3 | 6.3 | 4.3 | 82.0 | Chipman Chemical Company |
| Dritomic | 12.2 | 13.2 | 0.8 | 82.0 | General Chemical Company |
| Sulcoloid (1936) | 14.2 | 12.6 | 1.2 | 93.0 | Ansbacher Chemical Company |

[a] 1940 samples unless otherwise stated.

with the coarsest special sulphur and all of the copper samples (Table 1). Even if these values be multiplied by the usual shape-correction factor of $\frac{2}{3}$, agreement with the results of other methods is not good, since $d_1$ should be much less than $d_3$ with the finest copper zeolite. The very high figure obtained for $d_1$ may have been due to the fact that many of the finer translucent crystals of the zeolite were passed over in the counting. The photomicrograph of this material indicates that this might easily have been the case (Fig. 1).

To reduce the enormous amount of labor required to measure the particles in the finer materials, a method of treating the distribution count has been devised, so that only a relatively small number of particles need be counted (13, 17). By assuming that the Poisson size distribution occurs, and that the particles are spherical, a relatively simple calculation yields the

diameter of the particle of average weight (last column, Table 1). The results are in good agreement with those obtained.

Photomicrographs of spray residues of the different materials, when deposited as fine, evenly spaced, particulate drops upon glass slides, with a vertical sprayer, confirm the results of the particle size measurements. (*Cf.*

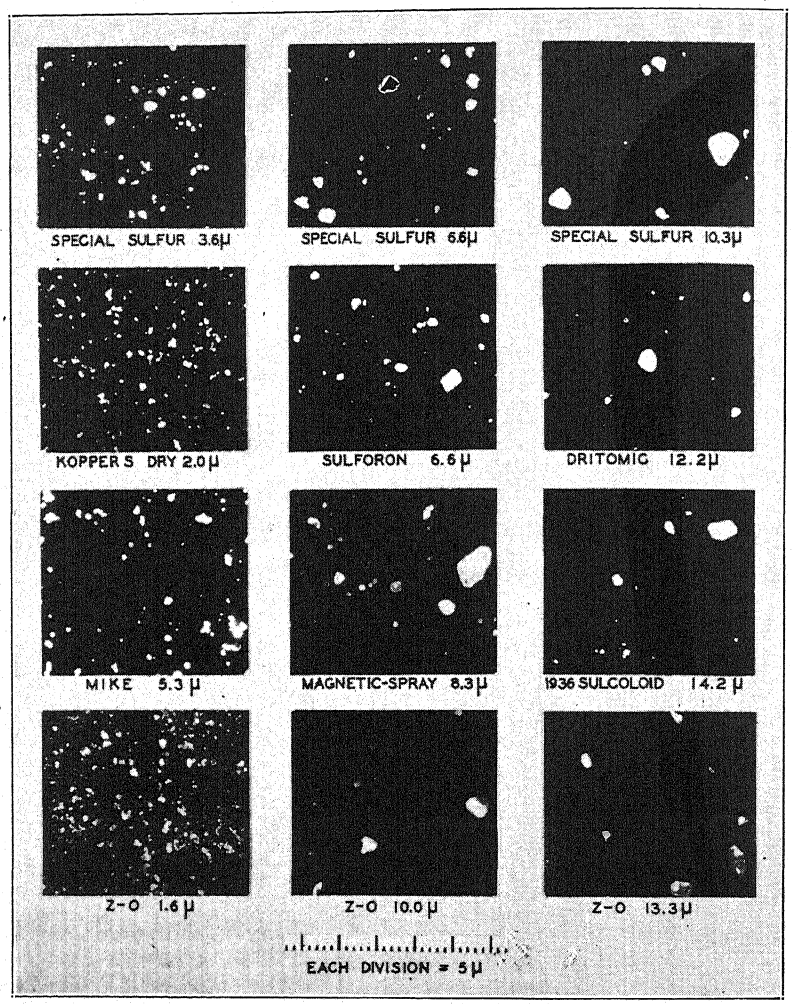



FIG. 1. Representative microscopic fields of sulphur and copper ammonium zeolite particles deposited upon glass slides in finely atomized even spaced particulate drops. The weight of all the particles in each area is the same. Duplicate slides were found by chemical analysis to contain $7.0 \pm 0.5$ micrograms of sulphur or copper ammonium zeolite per sq. cm.

Tables 1 and 2 with Fig. 1.) Since the fields of view are small, great care was taken to select a field truly representative of the material as a whole. A consideration of these photographs suggests that the larger average particle sizes result not so much from the presence of very large particles

as from the relatively small number of particles less than 4 or 5 microns in diameter.

While the differences between certain values have been discussed at length, it should be emphasized that the agreement between determinations in different laboratories on the materials of different ages and conditions of sampling still is remarkably close. From a practical point of view, a variation of 10 to 20 per cent in the average particle diameter of a wettable sulphur would scarcely be noticed in field experiments. Furthermore, the true particle size in water may not be the same as the effective particle size in an actual spray mixture. It is known that lime, lead arsenate, and many oil stickers flocculate sulphur particles into large agglomerates.

## STUDIES OF DISEASE CONTROL

### Orchard Experiments with Apple Scab

*Methods.* The experiments were conducted in mature, vigorous orchards in which the primary inoculum of apple scab was considered moderate to heavy. Single-tree plots were replicated from 2 to 6 times. A modern power sprayer was used, operated at 400 lb. pressure, with a single gun. The spray materials were washed, with the gun, through a screen into the tank. The same brand of arsenate of lead, which contained no wetting agent, was used in all the tests. At harvest, the scab-infected fruit was divided into 3 classes; slight, medium, and bad. Slight scab included those fruits that had scab spots affecting a total area up to $\frac{1}{4}$ inch in aggregate diameter, medium scab up to $\frac{3}{4}$ inch, and bad scab more than $\frac{3}{4}$ inch.

*Seasonal and Orchard Conditions.* Rainfall in the Hudson Valley varies considerably, not only from year to year but also from one locality to another in the same year. The amount of primary inoculum carried over in the old leaves also varies from orchard to orchard, depending on the previous disease history of the individual orchard. Therefore, it seems advisable to give a brief summary of the rainfall and inoculum facts concerned in these experiments (Table 3).

Series 1. Heavy primary inoculum and numerous prolonged rains made conditions ideal for a fungicide test. Some leaf infections occurred in all plots during bloom, and these became a source of secondary inoculum from which the trees had to be protected during the rest of the season. Fruits on nonsprayed Cortland trees became so heavily infected early in the season that they all dropped during June and July. Since all materials were applied at low concentrations, to accentuate any differences in the control of apple scab, it was necessary to apply 10 sprays instead of the 7 usually recommended.

Series 2. Primary inoculum and rainfall were considered average. Lack of protection during the abnormally long bloom period provided a source of secondary inoculum and a severe test for the fungicides during the rest of the season. Seven sprays were applied in this orchard.

Series 3 and 4. The orchard had only a moderate primary inoculum. The season was early and the trees passed through the first 4 spray applica-

tions with little chance for infection. Heavy rains occurred during the remainder of the season, and only the retentive materials were able to withstand this drastic washing.

Series 5. The orchard had a heavy carry-over of scab. Dry weather early in the season prevented development of scab on sprayed trees until July, but two heavy rains in August gave the fungus a chance to cause late fruit infection.

*Treatments and Results with Sulphur Materials.* The field experiments were designed primarily to determine the fungicidal values of the specially prepared sulphurs in which particle size was the only variable factor (Table 4, Ser. 1, 3, 5). Included, however, were commercial brands varying in particle size, but in which the effects are complicated by other factors such as shape of sulphur particles, differences in age of material, presence of adjuvants, and methods of adding wetting agents (Table 4, Ser. 2 and 4).

TABLE 3.—*Record of environmental factors that influenced apple scab development in experimental orchards of the Hudson Valley, 1937–1939*

| Year and series[a] | Location | Leaves with perithecia[b] | Rains | Seasonal rainfall[c] | Infection periods[d] |
|---|---|---|---|---|---|
| | | *Per cent* | *No.* | *Inches* | *No.* |
| 1937 Ser. 1 | Fishkill | 71 | 43 | 29 | 11 |
| 1937 Ser. 2 | Clermont | 24 | 16 | 14 | 9 |
| 1938 Ser. 3 and 4 | Rhinebeck | 38 | 35 | 24 | 9 |
| 1939 Ser. 5 | Modena | 80 | 19 | 4 | 5 |

[a] Series numbers identical to those in table 4.
[b] Percentage of overwintered leaves bearing perithecia. In each series, ascospores were mature by the delayed dormant stage of tree development.
[c] Rainfall, green-tip to harvest.
[d] Rain periods of sufficient duration for scab infection.

The data show, in every experiment, that the relative value of the wettable sulphurs in scab control was in inverse proportion to the average diameter of the sulphur particles. This all-important fact is true, not only for the special sulphurs but also for the commercial brands. Factors that may accentuate or obscure the differences in the degree of disease control obtainable with different particle-size sulphurs are seasonal weather conditions, inoculum potential, timing of sprays, varietal susceptibility, and concentration of toxicant.

In 1937, a wet year, commercial control of scab was obtained on McIntosh apples sprayed with 3.7 micron sulphur, while there was over 50 per cent scabby fruits in plots sprayed with 6.6 and 10.3 μ sulphur (Ser. 1). In 1939, a dry year, only the plot sprayed with the 10.3 μ sulphur failed to give commercial control (Ser. 5). The effect of particle size evidently is accentuated under conditions favorable for scab development.

Proper timing of spray applications tends to minimize the differences in scab control obtained with different particle-size sulphurs (Ser. 4). But in those orchards where scab had become established in the trees early in

the season, large differences in scab control were obtained with different particle-size sulphurs. (*Cf.* Ser. 1 and 2 with Ser. 3 and 4.)

Lowering the concentration of the sulphurs below the point where commercial control could be expected with the better materials further empha-

TABLE 4.—*The size of sulphur particles in relation to control of apple scab*

| Series, year, variety, and treatment | Average particle size of sulphur[a] | Total fruit | Percentage of scabbed fruits | | | |
|---|---|---|---|---|---|---|
| | | | Slight | Medium | Bad | Total |
| *Series 1*[b]—1937 | | | | | | |
| Delicious | | | | | | |
| Special sulphur 4–100 | 3.7 | 1277 | 3.8 | 3.7 | 2.8 | 10.3 |
| | 6.6 | 486 | 8.2 | 7.8 | 4.3 | 20.3 |
| | 10.3 | 1461 | 18.0 | 17.7 | 14.6 | 50.3 |
| Cortland | | | | | | |
| Special sulphur 4–100 | 3.7 | 3510 | 10.3 | 5.1 | 3.9 | 19.3 |
| | 6.6 | 3453 | 12.5 | 11.6 | 6.4 | 30.5 |
| | 10.3 | 2014 | 14.4 | 20.1 | 29.8 | 64.3 |
| McIntosh | | | | | | |
| Special sulphur 4–100 | 3.7 | 523 | 6.1 | 1.9 | 2.3 | 10.3 |
| | 6.6 | 268 | 18.2 | 9.3 | 26.5 | 54.1 |
| | 10.3 | 365 | 29.6 | 16.8 | 19.5 | 65.9 |
| *Series 2*—1937 | | | | | | |
| McIntosh | | | | | | |
| Nontreated | ..... | 7798 | 1.6 | 2.9 | 95.0 | 99.5 |
| Dow Mike 2–100 | 5.3 | 3427 | 15.4 | 10.3 | 3.2 | 28.9 |
| Magnetic-Spray 2–100 | 8.3 | 2954 | 19.3 | 21.3 | 12.0 | 52.6 |
| Dow Mike 4–100 | 5.3 | 4479 | 9.7 | 7.3 | 3.5 | 20.5 |
| Magnetic-Spray 4–100 | 8.3 | 3341 | 14.4 | 8.8 | 3.8 | 27.5 |
| Sulcoloid 4–100 | 14.2 | 3737 | 22.9 | 16.8 | 7.8 | 47.5 |
| *Series 3*—1938 | | | | | | |
| McIntosh | | | | | | |
| Nontreated | ..... | 3031 | 5.7 | 10.5 | 83.6 | 99.8 |
| Special sulphur 3–100 | 3.7 | 2609 | 4.3 | 3.5 | 3.6 | 11.4 |
| | 6.6 | 4318 | 5.4 | 9.6 | 9.2 | 24.2 |
| Special sulphur 5–100 | 3.7 | 6818 | 1.7 | 1.0 | 1.2 | 3.9 |
| | 6.6 | 4222 | 2.2 | 2.2 | 1.3 | 5.7 |
| | 10.3 | 5786 | 5.6 | 4.4 | 2.8 | 12.8 |
| *Series 4*—1938 | | | | | | |
| McIntosh | | | | | | |
| Nontreated | ..... | 3031 | 5.7 | 10.5 | 83.6 | 99.8 |
| Dow Mike 5–100 | 5.3 | 3941 | 1.4 | 1.4 | 1.6 | 4.4 |
| Magnetic-Spray 6–100 | 8.3 | 4413 | 2.7 | 4.9 | 1.8 | 9.4 |
| Sulcoloid 6–100 | 14.2 | 4665 | 3.3 | 6.1 | 3.0 | 12.4 |
| *Series 5*—1939 | | | | | | |
| McIntosh | | | | | | |
| Nontreated | ..... | 7045 | 12.3 | 13.7 | 66.0 | 92.0 |
| Special sulphur 4–100 | 3.7 | 2449 | 0.4 | 0.1 | 0.0 | 0.5 |
| | 6.6 | 9851 | 2.3 | 1.0 | 0.7 | 4.0 |
| | 10.3 | 8355 | 15.0 | 13.6 | 6.8 | 35.4 |
| Special sulphur 8–100 | 10.3 | 5415 | 11.8 | 6.5 | 3.7 | 22.0 |

[a] Surface average diameter.

[b] Nontreated trees in series 1 were heavily scabbed in June, and all fruits dropped at that time.

sized the greater effectiveness of the materials of smaller particle size (Ser. 2 and 3). Doubling the concentration of the 10.3 micron sulphur did not compensate for the greater toxicity of the 3.7 and 6.6 micron materials (Ser. 5).

## Greenhouse Experiments with Cedar-Apple Rust

*Methods and Conditions.* Potted Medina and Wealthy apple trees of comparable shoot development were selected for each experiment.

The trees were placed on a turntable and given a regulated application of a fungicide spray. These sprayed trees were then allowed to dry over night, and subsequently subjected to periods of artificial "rain" wash on the turntable (12).

For inoculation, the trees were atomized with standardized water suspensions of sporidia of *Gymnosporangium juniperi-virginianae.* Next, they were placed in a moist chamber for 16 hours at 16° C. and then returned to the greenhouse for the incubation period of at least 2 weeks.

Counts were made of the number of lesions on the 3 most heavily infected leaves per shoot, as the index to the effectiveness of the various fungicidal materials.

## Treatments and Results with Sulphur and Copper Materials

Summarization of greenhouse tests shows particle size to be the predominant factor in the fungicidal value of both the sulphur and the copper materials (Tables 5, 6, and 7).

The specially prepared sulphur, with different average particle size as the only variable factor, gave control of cedar-apple rust in inverse proportion to the size of the toxic particles (Table 5). A mixture of equal weights of the 3.7 and 10.3 μ sulphur was not far different in effectiveness from the 6.6 μ sulphur (Table 5, Ser. 2).

Ground copper zeolite, with average particle sizes of 1.6, 10, and 13.3 μ, exhibited fungicidal value in proportion to their relative degrees of fineness (Table 7).

Most of the commercial wettable sulphurs, also, adhered to this relationship between particle size and disease control (Table 6). Koppers Dry Wettable (2.0 μ), Micronized (3.6 μ), Sulforon (6.6 μ), Micro-Spray (6.7 μ), Kolospray (7.7 μ), Sulfix (8.8 μ), Microsulphur (10.3 μ), Apple Dritomic (12.2 μ), and Sulcoloid (14.2 μ) were effective in cedar-apple rust control about in that order. However, with Mike (5.3 μ), Magnetic-Spray (8.3 μ), and Kolofog (?), other factors in their composition seemed to obscure the particle size-disease control ratio. The shape of the particles in Mike sulphur and the thoroughness with which these are wet, due to the process of manufacture, may be factors influencing its tenacity and, consequently, its control. Unpublished data show that tenacity of wettable sulphurs tends to increase with age. In the case of Magnetic-Spray, electrical charges and a chemical used in the manufacturing process are claimed to be factors in its effectiveness, but these factors do not account entirely for the irregularities in disease control. Kolofog, a fused bentonite-sulphur containing sulphur particles of colloidal dimensions when dispersed in water, gave excellent control in this type of test (Ser. 5). However, under field conditions, Kolofog has not given consistent disease control presumably because the material

is either too rententive or has too low a sulphur content to be sufficiently redistributed.

### THE RELATIONSHIP BETWEEN PARTICLE SIZE, RETENTION, AND INHERENT TOXICITY OF SULPHUR AND COPPER MATERIALS

It has been shown that better control of apple scab and cedar-apple rust usually is obtained with the fungicides of smaller particle sizes. The question arises as to whether the increased control is due to a greater adherence of small particles to the leaves and fruit, to more thorough distribution, or to greater toxicity when proportionately larger surface areas of the toxic

TABLE 5.—*Cedar-apple rust control on potted Medina apple trees sprayed with sulphur of different average particle size*

| Series and treatment | Particle size[a] | Av. No. lesions on 3 heaviest infected leaves per shoot | |
|---|---|---|---|
| | | 2-in. wash[b] | 3-in. wash |
| *Series 1* | | | |
| Special sulphur 5–100 | 3.5 | 1 | 1 |
| | 3.7 | 1 | 6 |
| | 6.6 | 8 | 5 |
| | 10.3 | 100 ± | 100 ± |
| Unsprayed | ..... | 100 ± | ..... |
| *Series 2* | | | |
| Special sulphur 5–100 | 3.7 | 1 | 8 |
| | 6.6 | 30 | 37 |
| Special sulphur 2½–100 | 3.7 | 30 | 60 |
| " " 2½–100 | 10.3 | | |
| Unsprayed | ..... | 80 | ..... |
| *Series 3* | | | |
| Special sulphur 5–100 + S. E. C. Oil,[c] 1 pt. | 3.7 | 12 | 22 |
| | 6.6 | 20 | 23 |
| | 10.3 | 90 | ..... |
| Unsprayed | ..... | 300 ± | ..... |

[a] Surface average diameter.
[b] Inches of artificial rain on turntable.
[c] Self-emulsifiable cottonseed oil.

material are exposed. The relative adhesiveness was determined by analyses of the sulphur residues left on sprayed leaves. The effect of particle distribution and inherent toxicity was studied *in vitro* using the inhibition of germination of *Sclerotinia fructicola* spores as an indicator.

#### Sulphur Residue Analyses

*Methods.*—The sulphur residue analyses were made from leaf samples taken from sprayed orchard trees after various amounts of rainfall. The leaf samples were taken at random from the lower portions of three or more trees that had been sprayed as evenly as possible after terminal growth had stopped. As far as possible, leaves were selected from the same positions on the terminals. All samples for each series were collected by the same

person, to reduce sampling error to a minimum. Each sample lot consisted of 200 to 300 leaves. A 1-inch disc was cut from each leaf with a hollow steel punch. The discs were oven-dried, ground, and stored dry in fruit

TABLE 6.—*Cedar-apple rust control on potted Medina apple trees sprayed with commercial sulphur fungicides of different average particle size*

| Series and treatment | Particle size[a] | Av. No. lesions on 3 most heavily infected leaves per shoot |
|---|---|---|
| | | 0.5 in. wash[b] |
| *Series 1* | | |
| Micronized 5–100 | 3.6 | 4 |
| Sulforon 5–100 | 6.6 | 45 |
| Magnetic-Spray 5–100 | 8.3 | 23 |
| Sulcoloid (1936) 5–100 | 14.2 | 170 ± |
| Nontreated | ..... | 200 ± |
| *Series 2* | | |
| Micronized 7–100 | 3.6 | 4 |
| Mike 7–100 | 5.5 | 21 |
| Sulforon 7–100 | 6.6 | 19 |
| Magnetic-Spray 7–100 | 8.3 | 3 |
| Sulfix 7–100 | 8.8 | 24 |
| Microsulphur 7–100 | 10.3 | 60 |
| Nontreated | ..... | 130 ± |
| *Series 3* | | |
| Micronized 5–100 | 3.6 | 7 |
| Micro-Spray 5–100 | 6.7 | 13 |
| Sulforon 5–100 | 6.6 | 16 |
| Magnetic-Spray 5–100 | 8.3 | 22 |
| Sulfix 5–100 | 8.8 | 40 |
| Apple Dritomic 5–100 | 12.2 | 42 |
| Sulcoloid (1936) 5–100 | 14.2 | 130 ± |
| Nonsprayed | ..... | 300 ± |
| *Series 4* | | |
| Koppers Dry 7–100 | 2.0 | 7 |
| Micronized 7–100 | 3.6 | 23 |
| Mike 7–100 | 5.5 | 42 |
| Sulforon 7–100 | 6.6 | 32 |
| Magnetic-Spray 7–100 | 8.3 | 49 |
| Sulfix 7–100 | 8.8 | 61 |
| Apple Dritomic 7–100 | 12.2 | 150 ± |
| Sulcoloid (1936) 7–100 | 14.2 | 200 ± |
| Nontreated | ..... | 180 ± |
| *Series 5* | | |
| Micronized 5–100 | 3.6 | 10 |
| Micronized 2–100 | 3.6 | 49 |
| Kolospray 6–100 | 7.7 | 19 |
| Kolofog 6–100 | .....[c] | 1 |
| Nontreated | ..... | 100 ± |

[a] Surface average diameter.
[b] Inches of artificial rainfall on turntable.
[c] Particle size of Kolofog could not be satisfactorily determined due to the complexity of the material.

jars until ready for sulphur analyses. Two grams of the dried leaf sample were used for each determination.

The method of sulphur analyses is that of Emerson (4) as modified by White (18). This modified method has been found particularly suitable

for analyzing sulphur in spray residues. It should be noted that these results give the total sulphur on or in the leaf. It is not possible to distinguish between the sulphur that is no longer fungicidal and that that is still in the toxic form. However, it is sufficient for denoting the relative order of retention of the sulphur samples.

*Results.* Analyses of Rome Beauty leaves sprayed with the 3.5, 6.6, and 10.3 μ experimental sulphurs before and after successive rain periods showed particle size to be an important factor influencing tenacity (Table 8 and Fig. 2). In all instances, the original deposits decreased greatly with the first rain. No doubt the coarse sulphur particles, and those particles not in intimate contact with the leaves were removed with the first washing rain. After the first inch of rainfall, the rate of sulphur loss was much slower.

TABLE 7.—*Cedar-apple rust control of potted Medina apple trees sprayed with copper ammonium zeolite (Z–O) of three different average particle sizes*

| Series and treatment | Particle size[a] | Per cent of particles 4 microns and under | Max. lesions per sq. in. of leaf surface with given amounts of artificial rain | |
|---|---|---|---|---|
| | | | 0.5-in. wash[b] | 1.0-in. wash[b] |
| *Series 1*, Medina | | | | |
| Special Z–O 3–100 | 1.6 | 94.4 | 12 | 24 |
| | 10.0 | 5.4 | 47 | 69 |
| | 13.3 | 1.5 | 51 | 79 |
| Nonsprayed | ..... | ..... | 90 | ..... |
| *Series 2*, Wealthy | | | | |
| Special Z–O 3–100 | 1.6 | 94.4 | 6 | 15 |
| | 10.0 | 5.4 | 27 | 76 |
| | 13.3 | 1.5 | 42 | ..... |
| Nonsprayed | ..... | ..... | 150 ± | ..... |

[a] Surface average diameter.
[b] Inches of artificial rainfall on turnable.

It is distinctly evident that the sulphur of larger average particle size continues to be removed at a faster rate than that of smaller average size (Table 8 and Fig. 2). It is shown graphically that after 0.5-inch precipitation, there were retained 71, 68, and 61 per cent of the original deposits of the 3.5, 6.6, and 10.3 μ sulphurs, respectively; whereas, after 4.3 inches of rain, there were retained 53, 39, and 34 per cent in the same order.

Although these differences between the amounts of sulphur retained might seem small, unpublished data indicate that they are enough to make the difference between infection and control. They are believed to present a true picture of what takes place in normal spray practice.

It is worthwhile to note that the factor of particle size did not affect the amounts of the original deposits, a complicating factor often encountered in attempting to compare commercial brands of sulphur.

These analyses of fungicide-weathering from mature apple leaves in midsummer show a relatively slow loss of sulphur. Greenhouse studies and field

experience indicate that protection would be lost more rapidly during the spring growing season.

## Spore Germination Studies

*Methods.* To determine whether particle size affects the toxicity of sulphur and copper fungicides, a series of spore germination experiments were

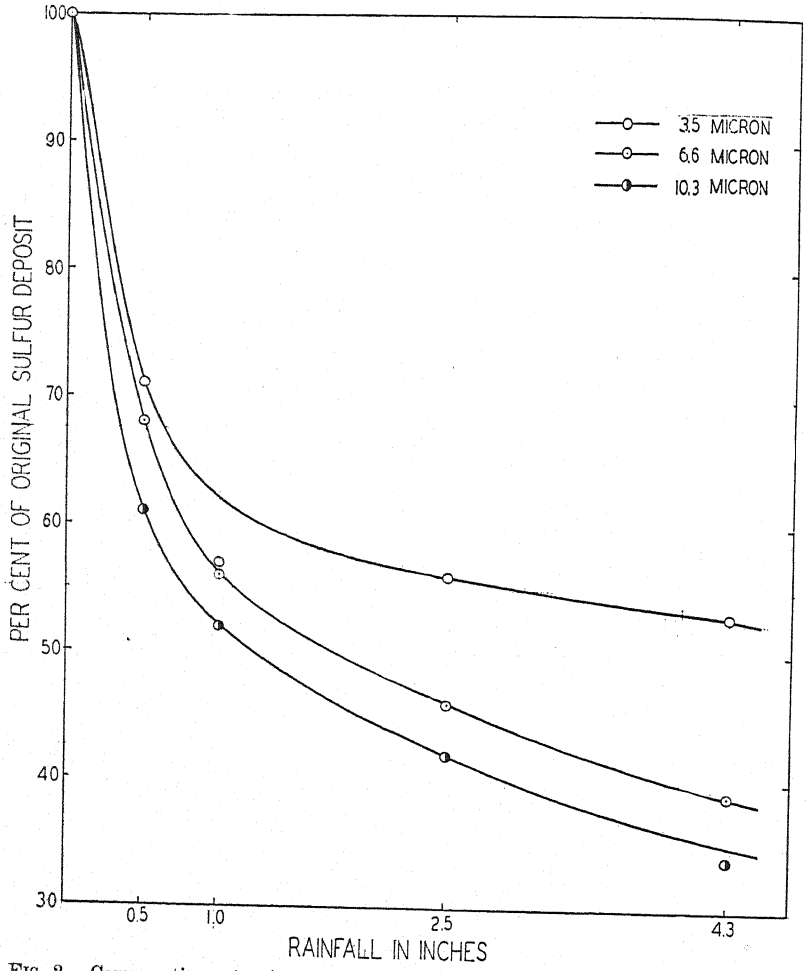



FIG. 2. Comparative retention of a special sulphur material of varying particle size to Rome Beauty foliage in the field.

made, using *Sclerotinia fructicola* (Wint.) Rehm as the test organism. The special wettable sulphur, in 3 particle sizes, was applied by a tower sprayer to glass slides coated with cellulose nitrate.[4] Copper zeolite, ground to 3 particle sizes, was sprayed on glass slides by means of a laboratory sprayer (14). *Sclerotinia fructicola* spores, from 7-day-old cultures, were suspended

[4] Hamilton, J. M., and G. L. Mack. A new vertical laboratory sprayer (in prep.).

in water containing synthetic media. (The synthetic media consisted of a stock solution of M/4 dextrose, M/5 $KNO_3$, M/20 $KH_2PO_4$, M/100 $MgSO_4$, and a trace of $FePO_4$.) This stock solution was diluted 800 times with redistilled water. The spore suspension was adjusted to a concentration of 60 spores per sq. mm. in a Fuchs-Rosenthal counting cell, and triplicate drops were placed on the slides with a standardized pipette. After incubation in moist chambers at a temperature favorable for germination, 100 spores were counted from each of 3 drops and averaged for the percentage of germination.

*Results.* The data show that the smaller the particle size, the greater the toxicity of the material (Tables 9 and 10). When the amount of fungicide deposited is plotted against the percentage of spores inhibited from germination by the fungicides, virtually straight parallel lines result for all materials.

TABLE 8.—*The comparative adhesiveness of fine, medium, and coarse sulphurs to foliage of Rome Beauty trees at successive intervals of rainfall*

| Special sulphur treatments | Date of collection | Rainfall in inches | Mg. of sulphur per g. of dried leaf | Percentage sulphur retained |
|---|---|---|---|---|
| | | | Av. of 2 samples | |
| 3.5 micron 4–100 | 7/20 | 0.0 | 2.74 ± .03 | 100 |
| | 7/27 | 1.02 | 1.80 ± .01 | 66 |
| | 8/12 | 3.92 | 1.31 ± .01 | 48 |
| 6.6 micron 4–100 | 7/20 | 0.0 | 2.71 ± .05 | 100 |
| | 7/27 | 1.02 | 1.37 ± .05 | 51 |
| | 8/12 | 3.92 | 1.22 ± .07 | 45 |
| 10.3 micron 4–100 | 7/20 | 0.0 | 2.79 ± .04 | 100 |
| | 7/27 | 1.02 | 1.17 ± .01 | 42 |
| | 8/12 | 3.92 | 0.82 ± .01 | 29 |

Photomicrographs of the inhibitory effect of sulphur materials of various particle sizes against the spores of *Sclerotinia fructicola* bear out the above assumption (Figs. 3 and 4). Under the conditions of these experiments, the relative length of the germ tubes is a better criterion of the inhibitory action of the sulphur than the percentage of the spores germinated. Commercial sulphur materials show the same trend in relation to particle size as the specially prepared samples in which particle size is the only variable factor (Fig. 4).

It might be assumed that differences in inhibition of spore growth were due to the fact that a given weight of small particles will cover the surfaces of the slides more thoroughly than the same weight of large particles and thus increase the chances of contact with the spores. However, an examination of the photomicrographs (Figs. 3 and 4) indicates that actual contact between spores and sulphur particles is no criterion of whether or not a given spore will germinate. While the mechanism of fungicidal action is not thoroughly understood, it seems logical to conclude that the finer particle materials were more toxic because of the larger surface area exposed to the water film in which the spores must germinate.

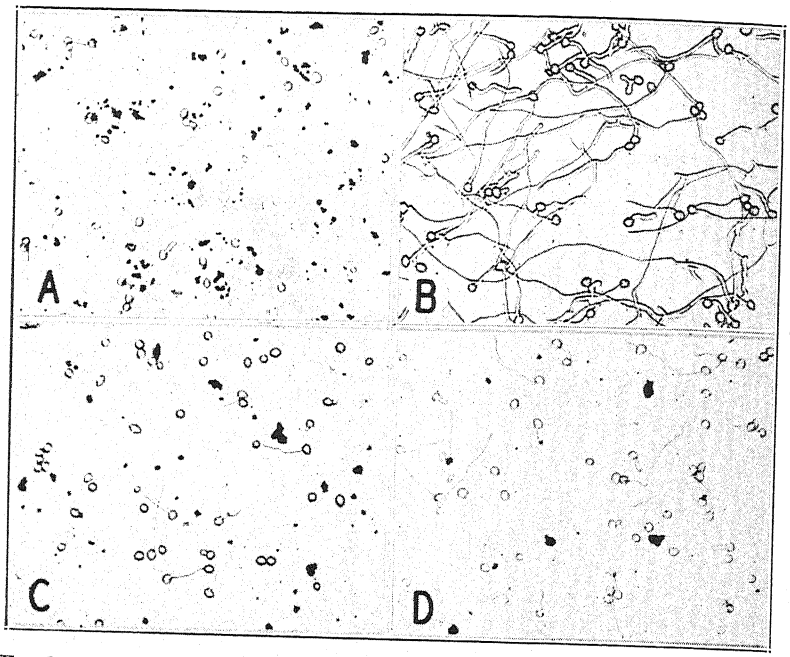



FIG. 3. The inhibition of spores of *Sclerotinia fructicola* grown *in vitro* in the presence of equivalent amounts of three grades of a special ground sulphur material varying only in particle size. Surface average diameter of the sulphur particles: A, 3.6 μ; C, 6.6 μ; D, 10.3 μ; B, control.

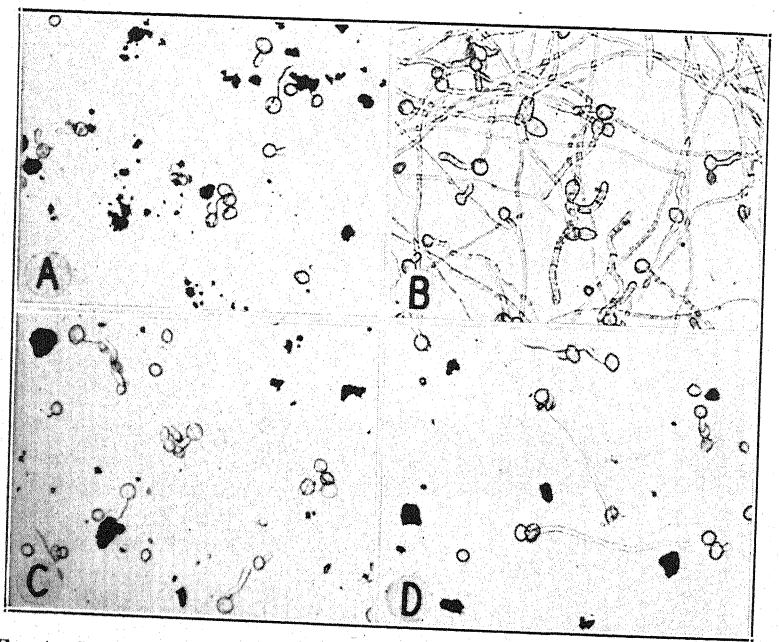



FIG. 4. The inhibition of spores of *Sclerotinia fructicola* grown *in vitro* in the presence of 7 micrograms per sq. cm. of commercial sulphur materials of various particle sizes. Surface average diameter: A, 3.6 μ; C, 6.6 μ; D, 8.8 μ; B, control.

TABLE 9.—*Toxicity of sulphur of three degrees of fineness to spores of Sclerotinia fructicola*

| Particle size in microns | Spray time in seconds | Per cent of spore germination inhibited |
|---|---|---|
| | | Average of 4 tests |
| 3.5 | 30 | 41 ± 1 |
| 3.7 | | 35 ± 1 |
| 6.6 | | 21 ± 2 |
| 10.3 | | 8 ± 1 |
| 3.5 | 60 | 48 ± 1 |
| 3.7 | | 43 ± 3 |
| 6.6 | | 31 ± 2 |
| 10.3 | | 16 ± 1 |
| 3.5 | 90 | 54 ± 2 |
| 3.7 | | 49 ± 2 |
| 6.6 | | 39 ± 2 |
| 10.3 | | 22 ± 2 |
| 3.5 | 120 | 58 ± 2 |
| 3.7 | | 53 ± 2 |
| 6.6 | | 46 ± 1 |
| 10.3 | | 28 ± 1 |
| Control | 0 | 3 ± 0 |

## SUMMARY

The fungicidal effectiveness of ground wettable sulphurs and an insoluble copper was found inversely proportional to the size of the particles. The correlation was established by experiments in the field, greenhouse, and laboratory.

A wettable sulphur and an insoluble copper, ground to 3 degrees of fineness to include the range of particle sizes found in most commercial mate-

TABLE 10.—*Toxicity of copper ammonium zeolite of three degrees of fineness to spores of Sclerotinia fructicola*

| Particle size in microns | Spray time in seconds | Copper deposit micrograms per sq. cm. | Per cent of spore germination inhibited |
|---|---|---|---|
| | | | Average of 2 tests |
| 1.6 | 3 | 0.7 | 27 ± 2 |
| 10.0 | | | 25 ± 1 |
| 13.3 | | | 2 ± 0 |
| Commercial Z–O | | | 2 ± 1 |
| 1.6 | 8 | 1.8 | 42 ± 1 |
| 10.0 | | | 39 ± 2 |
| 13.3 | | | 10 ± 2 |
| Commercial Z–O | | | 12 ± 2 |
| 1.6 | 13 | 2.9 | 60 ± 2 |
| 10.0 | | | 54 ± 2 |
| 13.3 | | | 33 ± 2 |
| Commercial Z–O | | | 28 ± 1 |
| 1.6 | 20 | 4.4 | 81 ± 1 |
| 10.0 | | | 73 ± 2 |
| 13.3 | | | 57 ± 2 |
| Commercial Z–O | | | 52 ± 2 |
| Control | 0 | 0.0 | 2 ± 1 |

rials, were tested against apple scab, *Venturia inaequalis* (Cke.) Wint., apple rust, *Gymnosporangium juniperi-virginianae* Schw., and brown rot, *Sclerotinia fructicola* (Wint.) Rehm. Similar experiments were conducted with commercial wettable sulphurs whose fungicidal properties were affected not only by particle size but also by the presence of various adjuvants, and by different methods of manufacture. Sulphur materials prepared by the Grinrod and flotation processes were relatively less adhesive than ground sulphur of equal particle size, provided no material was added to aid retention.

The particle-size measurements were checked by 4 different methods and fair agreement was obtained. The variation in results obtained by different methods is discussed and it is concluded that the Andreasen sedimentation method and the air-permeation method are the most reliable procedures.

Photomicrographs of the particles in representative materials further confirmed the results of the particle-size determinations. These pictures emphasize the fact that for a given weight of material there are many more particles per unit area for a fine powder than for a coarse one.

## LITERATURE CITED


1. ANDREASEN, A. H. M., W. JENSEN, and J. J. V. LUNDBERG. Ein Apparat für Dispersoidanalyse und einige Untersuchungen damit. Kolloid-Zeitschr. 49: 253–265. 1929.
2. BLODGETT, F. M. Hop Mildew. N. Y. (Cornell) Agr. Exp. Stat. Bull. 328: 277–312. 1913.
3. CARMAN, P. C. The determination of the specific surface of powders. Part I. J. Chem. Soc. Ind. 57: 225–234. 1938. Part II. J. Chem. Soc. Ind. 58: 1–7. 1939.
4. EMERSON, H. Micro-determination of sulphur by fusion. Jour. Amer. Chem. Soc. 52: 1291–1292. 1930.
5. GOODEN, E. L. A powder compactor for air-permeation experiments. Ind. Eng. Chem., Anal. Ed. 13: 483–484. 1941.
6. ————. Commercial insecticidal sulfurs. Average particle diameters as determined by air permeation. Ind. Eng. Chem. 33: 1452–1453. 1941.
7. ————, and C. M. SMITH. Measuring average particle diameter of powders. An air-permeation apparatus. Ind. Eng. Chem., Anal. Ed. 12: 479–482. 1940.
8. GREEN, H. The effect of non-uniformity and particle shape on "average particle size." Journ. Franklin Inst. 204: 713–729. 1927.
9. HAMILTON, J. M. Studies on apple scab and spray materials for its control in the Hudson Valley. N. Y. (Geneva) Agr. Exp. Stat. Tech. Bull. 227. 1935.
10. ————. Recent investigations on the control of cedar-apple rust in the Hudson Valley. N. Y. (Geneva) Agr. Exp. Stat. Bull. 678. 1937.
11. ————, and D. H. PALMITER. Apple Scab Control in 1937. Proc. New York State Hort. Soc. 83: 22–29. 1938.
12. ————. Studies of the fungicidal action of certain dusts and sprays in the control of apple scab. Phytopath. 21: 445–523. 1931.
13. HEUBERGER, J. H., and J. G. HORSFALL. Relation of particle size and color to fungicidal and protective value of cuprous oxides. Phytopath. 29: 303–321. 1939.
14. HORSFALL, J. G., J. H. HEUBERGER, E. G. SHARVELLE, and J. M. HAMILTON. A design for laboratory assay of fungicides. Phytopath. 30: 545–563. 1940.
15. MCMILLEN, E. L. The adhesion-tension cell in paint investigations. Ind. Eng. Chem. 22: 890–893. 1930.
16. STREETER, L. R., and W. H. RANKIN. The fineness of ground sulfur sold for dusting and spraying. N. Y. (Geneva) Agr. Exp. Stat. Tech. Bull. 160. 1930.
17. THORNDIKE, F. Applications of Poisson's probability summation. Bell System Tech. Jour. 5: 604–624. 1926.
18. WHITE, R. P. Studies on the adhesiveness of sulphur residues on foliage. N. J. Agr. Exp. Stat. Bull. 611. 1936.
19. WILCOXON, F., and S. E. A. MCCALLAN. Fungicidal action of sulphur. III. Physical factors affecting the efficiency of dusts. Contrib. Boyce Thompson Inst. 3: 509–528. 1931.

# REACTION OF RESISTANT TOBACCOS TO CERTAIN STRAINS OF NICOTIANA VIRUS 1 AND OTHER VIRUSES[1]

H. H. McKinney[2]

(Accepted for publication November 8, 1942)

## INTRODUCTION

Data presented in another paper (13) show that certain collections of tobacco from Colombia, which are exceedingly unfavorable hosts for Nicotiana virus 1, rarely manifest gross signs of disease when infected experimentally. However, when these highly resistant plants are infected with certain yellow-type mutants from Nicotiana virus 1, symptoms may or may not result, depending on the conditions of culture. Tests were carried out with several virus mutants isolated by the writer, with several varieties or strains of Nicotiana virus 1, collected in the field by the writer and others, and with several viruses outside the common-mosaic group.[3]

## MATERIALS AND METHODS

The plant materials employed in the research herein reported were described in another paper (13). They comprised 3 tobaccos, *i.e.*, 1. Wisconsin-Havana Seed, very susceptible; 2. Ambalema, resistant; 3. T.I. 448A, very resistant; and 4. *Nicotiana glauca* L. (8).

Most of the assays were made on the primary leaves of *Phaseolus vulgaris* L., var. Scotia, in accordance with the methods outlined previously (13), but other testing species were used for certain viruses as indicated throughout the paper. In certain of the assays, 600-grain carborundum powder was dusted on the bean leaves before wiping with the extracts.

Those viruses used were: Nicotiana virus 1 and seven distinct mutants isolated from Nicotiana virus 1. Although none of these mutants can be regarded as slow-moving in Wisconsin-Havana Seed tobacco, they constitute a graded series for the degree of chlorosis incited, beginning with the mild-green mosaic mutant *AMG* (12), and ending with the severe yellow-mosaic mutant *BSY* (12).[4] "White" mosaic virus[5] collected in the field by W. D.

[1] Studies supported in part by funds provided under Bankhead-Jones Project S.R.F. 2–17, Agricultural Research Administration, U. S. Department of Agriculture. These studies were cooperative between the Bureau of Plant Industry and the Bureau of Agricultural Chemistry and Engineering.

[2] Senior Pathologist, Division of Cereal Crops and Diseases. Acknowledgment is due Matthew Koerner for assistance in conducting the tests; W. D. Valleau, E. E. Clayton, S. P. Doolittle, E. S. Schultz, and T. P. Dykstra for supplying virus or other materials.

[3] Smith's (18) system of nomenclature is used for those viruses he has listed.

[4] The term yellow mosaic as used by the writer has always been based on the pigment coloration in dark tobacco. When mutant *BSY* was isolated in 1926, the writer then attempted, without success, to isolate a virus that might induce a bleached or white mottling. Since then he has tried several times to obtain such an isolation, but without success. When the writer's yellow-mosaic *BSY* was studied on light-green tobaccos of the Burley type, white mosaic resulted. The normal plastid pigment content of these tobaccos is so low in comparison with that in the dark tobaccos, that the yellow pigments frequently reach their end points in a large percentage of the mottled tissue. Since all of the research on isolated yellow-mosaic mutants has been done with the dark green tobaccos, and since

Valleau (19); Plantago-mosaic virus collected by the writer, and similar to *Marmor tabaci* var. *plantaginis* described by Holmes (5); Type *B* yellow-mosaic virus (9); Nicotiana virus 6 (12); Type *C* yellow-mosaic virus (9); Solanum virus 2, veinbanding-mosaic source; Solanum virus 2, "Y"-mosaic source; Cucumis virus 1, cucumber-mosaic source; Cucumis virus 1, celery-mosaic source, and Nicotiana virus 12 (tobacco ring spot).

The experiments were conducted in a greenhouse and in controlled-temperature chambers. All tests were made in daylight. The methods of culture were the same as those previously described (13).

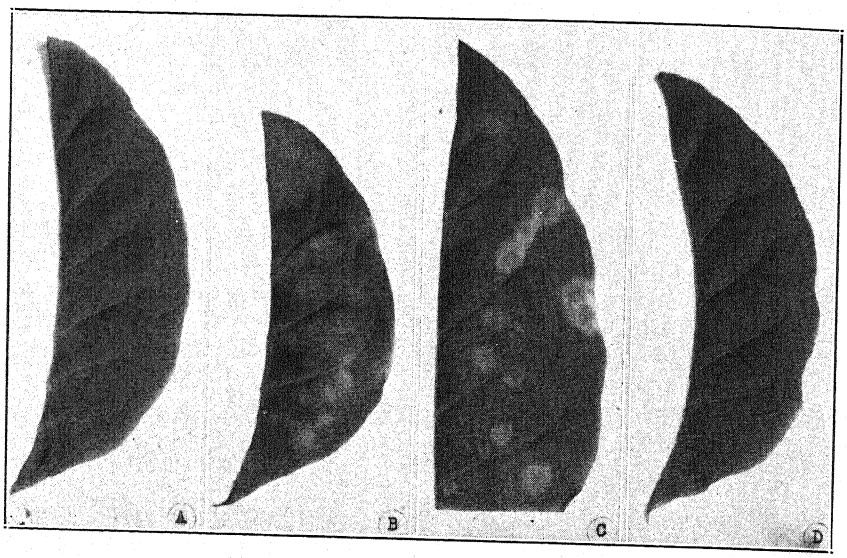



FIG. 1. Portions of leaves of tobacco T.I. 448A illustrating the influence of age of plants on the expression of symptoms when yellow-mosaic mutant *BSY* is used for the inoculum. A. Healthy control. B. Wiped half-leaf of very young plant showing yellow primary spots. C. Half-leaf from same plant as (B) and above it showing yellow spots from systemic virus. D. Half-leaf from a plant 3 weeks older but inoculated from the same virus extract and at the same time as the very young plant. This leaf contained virus.

## EXPERIMENTAL RESULTS

### Reaction of T.I. 448A to Some of the Mutants from Nicotiana Virus 1

Several preliminary tests were carried out in the greenhouse with plants of T.I. 448A, ranging from 40 to 80 days old from date of seeding. It was found that the mild-green mosaic mutant *AMG* induced no symptoms under any conditions, though virus was isolated from the inoculated plants. In young plants 40 days old, all 6 of the yellow-mosaic mutants that were tested

green dark tobaccos have been for so long used and by so many workers, it is evident that if light tobaccos are employed this fact should be so stated by the experimenter. Otherwise, there will be resultant confusion.

[5] The term "virus" as applied here and elsewhere in this paper, signifies no virus of specific rank, but merely an infectious entity. This usage compares with that of such terms as pathogen, parasite, infectious agent, fungus, bacterium, etc. Throughout the paper this usage has been made clear by the context.

induced primary and secondary yellow spots, but in plants 49 days old, and older, irregularities became more evident; 3 of the yellow-mosaic mutants failed to induce secondary symptoms; primary symptoms were faint and sometimes absent. Figures 1 and 2 illustrate resistance coincident with age and genotype.

These results made it very clear that some of the mutants were less infective than others and that other factors were operating. It appeared that

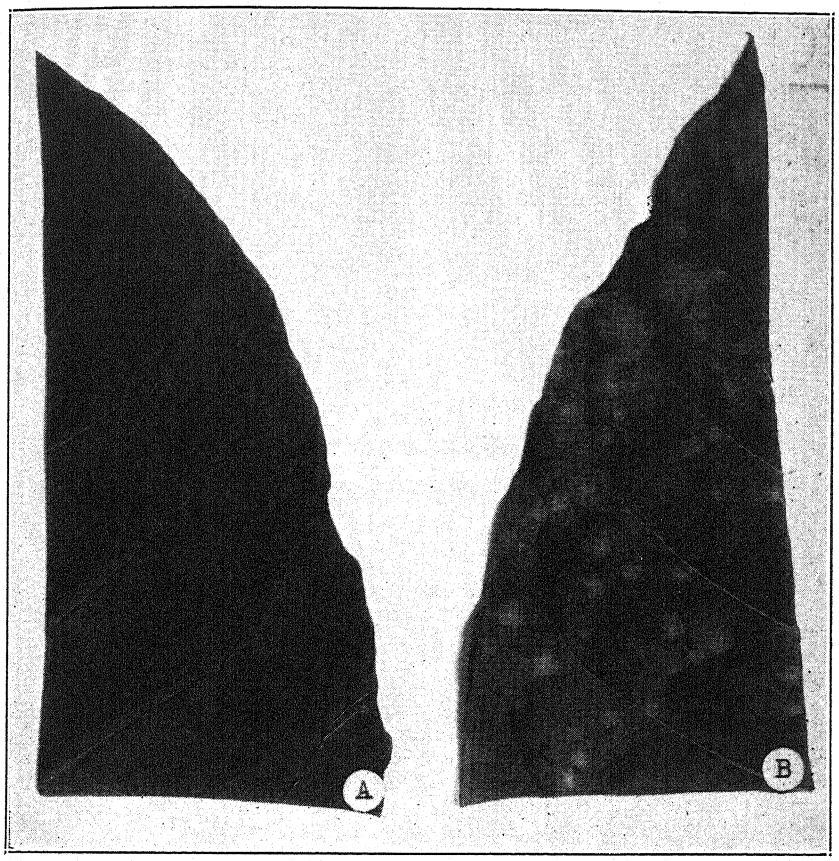



FIG. 2. Portions of leaves from large tobacco plants wiped with yellow-mosaic mutant *BSY*. A, T.I. 448A; B, Wisconsin-Havana Seed.

age of plant and temperature are important. As mutant *BSY* (12) seemed to be less erratic than some of the other yellow-mosaic mutants, it was used in much of the subsequent work.

*Effect of Age of Plants.* In young plants inoculated with *BSY* by the leaf-wipe method, and cultured near 22° C., secondary chlorotic spots developed on 7 to 17 leaves above the inoculated leaves. The number of chlorotic spots and their intensity in yellow color decreased on the leaves progressively up the plants. Secondary chlorotic spots frequently developed a

concentric pattern (Fig. 1 C). In comparison with the highly susceptible control plants, the primary chlorotic spots usually appeared from 1 to 3 days later on the wiped leaves of T.I. 448A.

The first signs of secondary chlorotic spots usually appeared at the tip and margins of leaves that were $\frac{3}{4}$ to $1\frac{1}{4}$ inches long when the plant was inoculated. As these leaves enlarged, new spots appeared progressively towards the petioles; and there was considerable spread of chlorosis from the original centers, especially near the apical margins of the leaf. Several subsequent leaves developed chlorotic spots in the same manner; but, since the number of spots per leaf became progressively less in subsequent leaves,

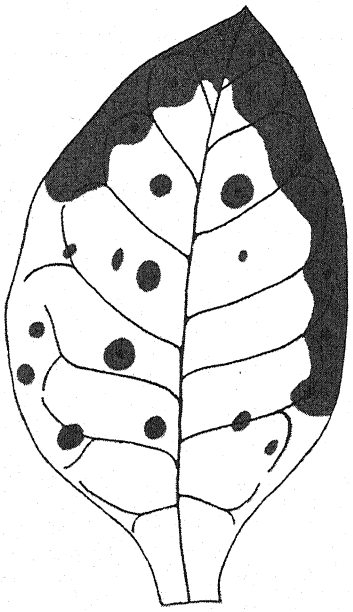


FIG. 3. Diagram of leaf from young plant of T.I. 448A (third above the inoculated leaf); virus of yellow mutant *BSY* used; the darkest spots denote the initial, discrete yellow spots which appeared; the gray spots and areas denote spots which appeared later and also the spread of chlorosis from yellow centers.

the sequence of their appearance from the tip to the base of the leaf became less striking. By far the majority of chlorotic spots was centered in the mesophyll traversed by the smallest veins. Very few spots had their centers in the veins, and most of these appeared in the marginal region where the veins are small (Fig. 3).

Unlike the mosaic mottling of Wisconsin-Havana Seed tobacco, the chlorotic spots did not appear on the very small central leaves at any stage of the plant's development.

Eight plants of T.I. 448A, 66 days from seedling, were inoculated with fresh virus extract of *BSY*. Four of these plants were inoculated by means of needle punctures in the leaf axils near the growing tip and four were inoculated by wiping nondiluted virus extract on 3 leaves.

No symptoms appeared in and no virus was recovered from the plants inoculated by means of the needle. Local chlorotic spots appeared on all the leaves in the wiped series, one plant developed secondary symptoms on 5 leaves and 3 plants failed to show secondary symptoms.

A plant of T.I. 448A and a plant of Wisconsin-Havana Seed, each about 80 days old, were inoculated by wiping the leaves with nondiluted fresh extract of *BSY*. No local chlorotic spots appeared on the wiped leaves of T.I. 448A, but from 50 to 400 spots appeared on the wiped leaves of Wisconsin-Havana Seed (Fig. 2). When the primary symptoms appeared on Wisconsin-Havana Seed tobacco, one-half of each of 11 leaves from each of the varieties was assayed. The leaf samples were thoroughly pulped in an equivalent amount of buffered solution by weight and the resulting extracts, without further dilution, were wiped on Scotia bean leaves. The extracts from the leaves of Wisconsin-Havana Seed induced from 212 to over 500 lesions per bean plant. Four of the leaf samples from T.I. 448A failed to induce lesions, 6 failed to induce more than a trace, which might represent residual virus, and 1 leaf induced an average of only 12 lesions per bean plant. Twelve days later the remaining halves of leaves on the T.I. 448A plant were assayed. The highest level of activity was in a sample that induced 41 lesions per bean plant.

When the leaves of large plants of T.I. 448A were dusted with 600-grain carborundum powder and wiped with strain *BSY*, local chlorotic spots appeared in large numbers. In one test, the effect of carborundum was determined on half leaves. The number of local spots on the halves receiving no carborundum ranged from 0 to 10, and on those receiving the powder, the number ranged from 172 to over 300. The latter figure could not be determined accurately as the spots were too numerous.

Assays indicate that mutant *BSY* is not present in the upper portion of mature infected plants of T.I. 448A. This virus-free zone seems just as extensive, if not more so, when *BSY* is applied, as is the case when Nicotiana virus 1 is used (13).

In young plants of T.I. 448A, mutant *BSY* has not been isolated from the growing points and smallest tip leaves when the typical secondary symptoms obtain in the large leaves. In one test, a group of plants showing typical symptoms was used for making virus assays on extracts from the young symptomless leaves. These leaves were divided into 3 groups as follows: (1) growing points and tip leaves 15 mm. long and shorter; (2) leaves next below (1) and 22 to 35 mm. long; and (3) leaves next below (2) and 50 to 65 mm. long. These samples were finely pulped in 4 parts of buffered solution and wiped on bean leaves previously dusted with carborundum powder. No necrotic lesions were induced by any of the extracts. In similar tests, traces of virus have been detected occasionally in leaves 50 to 65 mm. long, but virus is rarely detectable in leaves 30 to 40 mm. long.

Since carborundum powder greatly increases the number of primary infection sites in T.I. 448A, it was thought that typical mosaic mottling

might occur if concentrated virus extracts were wiped on all the leaves of plants dusted with carborundum powder. However, in spite of the myriads of primary sites that developed, there was not a single case of mosaic mottling in 60 T.I. 448A plants so treated.

In susceptible tobacco such as Turkish, variety Samsun, and Wisconsin-Havana Seed, *BSY* has been detected without fail in the growing points and smallest leaves from plants showing the typical yellow-mosaic symptoms, even when the samples were diluted to $10^{-4}$ with buffered solution. In such a test, run concurrently with that cited in the preceding paragraph, the growing points and small mottled leaves 6 mm. long contained sufficient virus to induce 124 lesions per bean plant, and mottled leaves, 35 to 40 mm. long, from the same plants, induced 400 lesions per bean plant.

*Effect of Temperature.* Experimental consideration was given to the effect of temperature on infection and on expression of symptoms in T.I. 448A and Ambalema plants inoculated with Nicotiana virus 1 and its mutant *BSY*. Plants 64 days old were used. Counting tip leaves $\frac{1}{2}$ inch long, the plants bore 8 to 9 leaves each. Three well-developed green leaves were wiped with nondiluted fresh virus extracts. Three plants of each combination of virus and variety were placed in each of 3 temperature chambers controlled near 22.5° C., 28.5° C., and 33.5° C., respectively. Winter daylight was the only source of illumination.

The results are given in table 1, and samples of the leaves wiped with strain *BSY* are illustrated in figure 4. Leaves wiped with Nicotiana virus 1 are not illustrated, as they developed no symptoms. The necrotic lesions induced by *BSY* on Ambalema at 28.5° C. (Fig. 4, E) were very small. They started as minute brown points resembling those at 22.5° C. (Fig. 4, D); but, unlike the latter, they made more progress, and developed light-green to yellow margins. On the leaves of T.I. 448A, no necrotic lesions appeared at any of the temperatures, but diffuse yellow zones did develop at 28.5° C. and 33.5° C. (Fig. 4, A, B, C).

It is of interest to note that, in Ambalema at 22.5° C. and 28.5° C., no secondary cholorotic spots appeared, and assays failed to reveal systemic virus in these plants. At these same temperatures, some of the plants of T.I. 448A manifested secondary chlorotic spots. The tendency for Ambalema to produce only local necrotic lesions at the lower temperatures and to develop secondary symptoms at the highest temperature is similar to, although not identical with, the situation obtaining in *Nicotiana sylvestris* L. when inoculated with *BSY* (17), designated in that paper as yellow-mosaic virus (type *A*).

It will be observed in table 1, that Nicotiana virus 1 induced a few secondary light-green spots and oak-leaf patterns in both T.I. 448A and Ambalema cultured at 33.5° C. These symptoms involved but 1 to 3 leaves on each plant. The oak-leaf patterns consisted of rather faint outlines having the general form of the patterns induced on old leaves of Wisconsin-Havana Seed tobacco infected with some of the yellow mosaics. The writer illustrated this reaction in an early paper (8, Fig. 8).

TABLE 1.—*Temperature in relation to the expression of symptoms in young plants of T.I. 448A and Ambalema tobacco. Tests conducted during December and January*

| Virus used | Symptoms at temperatures and on varieties indicated | | | | | |
|---|---|---|---|---|---|---|
| | 22.5° C. | | 28.5° C. | | 33.5° C. | |
| | T.I. 448A | Ambalema | T.I. 448A | Ambalema | T.I. 448A | Ambalema |
| Nicotiana virus 1 | No primary symptoms<br>No secondary symptoms | No primary symptoms<br>No secondary symptoms | No primary symptoms<br>No secondary symptoms | No primary symptoms<br>No secondary symptoms | Very few primary light-green spots<br>Very few secondary light-green spots and "oak-leaf" patterns on a few leaves of 3 plants | Very few primary light-green spots<br>Very few secondary light-green spots and "oak-leaf" patterns on a few leaves of 3 plants |
| Yellow-mosaic mutant *BSY* | No primary symptoms<br>Secondary chlorotic spots on 11 leaves on each of 2 plants; 1 plant free | Many small brown pin-point necrotic spots on all wiped leaves<br>No secondary symptoms | Light-green to yellow spots on all wiped leaves<br>Secondary chlorotic spots on 8 leaves of 1 plant; 2 plants free | Many small necrotic lesions and pin-point spots on all wiped leaves. Light-green margins around lesions<br>No secondary symptoms | Many large yellow spots on all wiped leaves<br>Secondary yellow chlorosis, which gradually spread over most of the surface of 3 leaves on 1 plant. Two plants free | Many large yellow spots on all wiped leaves<br>Secondary yellow chlorosis, which gradually spread over most of the surface of 3 leaves on 3 plants |

Mutant *BSY*, at 33.5° C. did not induce primary chlorotic spots of a discrete type in either T.I. 448A or Ambalema. Instead, diffuse yellow

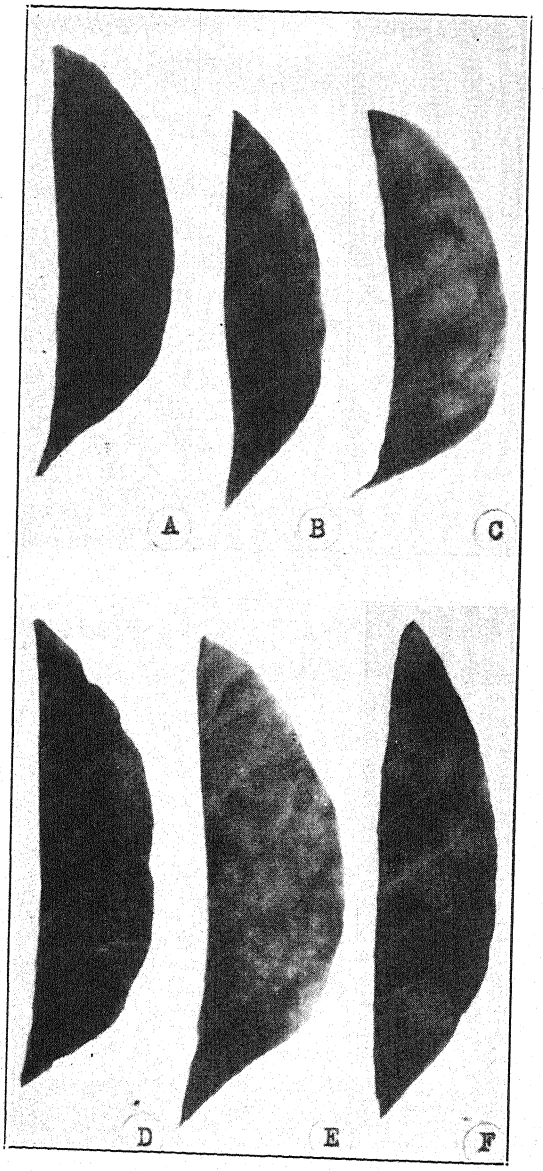



FIG. 4. Portions of leaves illustrating the influence of temperature on primary symptoms in young plants. A, B, and C, from T.I. 448A, incubated at 22.5° C., 28.5° C., and 33.5° C., respectively; D, E, and F, from Ambalema incubated at 22.5° C., 28.5° C., and 33.5° C., respectively.

patches appeared near the bases of the leaves and along the midrib and large veins. This chlorosis took on the form of the oak-leaf pattern, but this

effect was obscured as chlorosis finally involved most of the surface of a leaf. This reaction occurred in 3 leaves on each plant. It was impossible to continue this test through the entire vegetative period.

In another test, with mutant *BSY*, plants of T.I. 448A, but younger than those referred to in table 1, developed chlorotic spots on wiped leaves at temperatures near 23° C. Younger plants also have shown secondary symptoms in a more severe form at temperatures near 28.5° C. than was manifested at this temperature in the above test. However, even very young plants of T.I. 448A sometimes fail to manifest secondary symptoms. In this test 21 small plants of T.I. 448A were inoculated with concentrated virus extract of *BSY;* 3 to 4 leaves on each plant were wiped; virus also was introduced into the stem tip of each plant by means of a needle. The culture temperature was near 23° C. Secondary chlorotic spots appeared in 10 leaves on 4 plants; in 8 to 9 leaves on 9 plants; in 6 to 7 leaves on 6 plants; and in 2 leaves on 1 plant. In one plant there were no secondary symptoms. There is no evidence that the plants manifesting these different reactions and nonreaction represent distinct genotypes. The variability is explained on the basis that T.I. 448A is a poor suscept, in which case disease expression is subject to relatively slight differences in conditions that would not be reflected in highly susceptible plants.

## Localization of Nicotiana Virus 1 and Its Mutant *BSY* in T.I. 448A

*Tests with Mutant BSY.*—Preliminary data and observataions suggested that the major increase of virus in T.I. 448A is confined to relatively few scattered zones, and as previously reported (10), this was found to be the case.

One of the first leaves of T.I. 448A to manifest severe secondary chlorotic spotting from mutant *BSY* was removed, and all spots were carefully located on a sketch illustrated in figure 3. Assays were made from (1) the yellow tissue, (2) the normal green tissue, (3) the large lateral veins, and (4) the midrib. All green tissue, lateral veins, and midrib tissue adjoining the yellowish-green margins of the yellow tissue were discarded. From 2 to 3 mm. of normal green tissue was left attached to and assayed with the lateral-vein tissue. The tissues were pulped thoroughly in 3 parts of buffer by weight, and the extracts were wiped on the primary leaves of Scotia bean plants. The lesion counts for the 4 tissues are listed in table 2.

TABLE 2.—*Virus concentration in yellow tissue and in green tissue of leaf of T.I. 448A tobacco infected with mutant BSY as shown by assays on Scotia bean plants. Diagram of leaf shown in figure 3*

| Tissue tested | Lesions per plant |
|---|---|
| Yellow zones | 701.3 |
| Green lamina, no signs of chlorosis | 48.0 |
| Lateral veins | 46.7 |
| Mid-veins | 0.3 |

These data indicate that the virus is more abundant in, but not confined to the yellow zones. Since the counts for the lateral veins and for the green tissue are so close, it seems reasonable to conclude that the bulk of the virus in the vein sample was in the attached green tissue. The low count from the midrib seems to indicate very little virus in the large vascular channels.

Another leaf bearing a single yellow spot, was cut into sections and each section thoroughly pulped in 0.5 cc. of buffered solution and assayed on bean leaves. The number of lesions per bean plant induced by each section of leaf tissue is given in figure 5. It will be observed that all detectable virus was located in and around the yellow spot, and that part of the normal green tissue near the yellow spot showed slightly greater virus activity than the yellow spot.

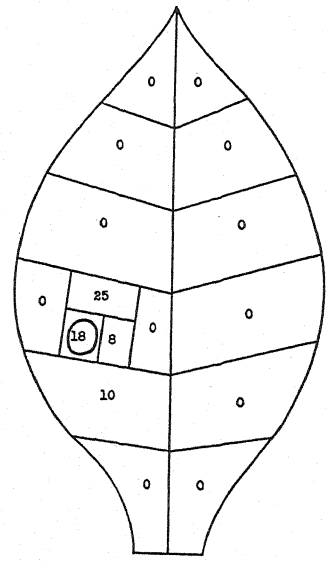



FIG. 5. Diagram of young leaf from plant of T.I. 448A, second above leaf inoculated with virus of yellow mutant *BSY*. Circle denotes a yellow spot; all sections of leaf outlined were assayed for virus; numerals denote number of lesions per bean plant.

A plant of T.I. 448A infected with mutant *BSY* exhibited chlorotic spots on 4 consecutive leaves. The 8 leaves above these appeared normal in every way. The 4 spotted leaves and 4 apparently normal ones immediately above were assayed separately, each on 4 bean plants. Extracts from entire leaves were expressed from fine-ground tissue without dilution. The results in table 3 show that detectable virus is not confined to leaves exhibiting the yellow spots.

*Test with Nicotiana Virus 1.*—A test was carried out to determine the systemic distribution of Nicotiana virus 1 in a leaf of T.I. 448A. Part of a leaf 22 cm. long was removed from an infected plant. Preliminary assays on parts of this leaf had shown the presence of virus, but there were no visible symptoms. The portion used in this test was cut into 19 sections con-

forming to the main vascular channels and leaf lamina (Fig. 6). Each section was finely ground in 3 parts of buffered solution, and the extracts were wiped on bean leaves. The numerals in the sections in figure 6 indicate the number of necrotic lesions per bean plant induced by the extracts from the sections. It will be observed that nearly all of the detectable virus occurred in 3 adjacent sections of laminal tissue.

The data cited in this chapter show that Nicotiana virus 1 and mutant *BSY* are localized to a very high degree in T.I. 448A tobacco. *BSY* virus tends to be most concentrated in the chlorotic zones, but it is not confined to them. This observation is at variance with the results reported by Valleau and Diachun (19). They succeeded in isolating virus only from the yellow zones in resistant tobacco leaves infected with their "white"-mosaic virus.

TABLE 3.—*Tests for presence of mutant BSY in spotted and adjacent nonspotted leaves of T.I. 448A tobacco, as shown by assays on Scotia bean plants. Extracts not diluted*

| Leaf No.[a] | Yellow spots on leaf | Lesions per plant |
|---|---|---|
| | *Number* | *Number* |
| 10 | 12 | 361 |
| 11 | 6 | 127 |
| 12 | 20 | 216 |
| 13 | 5 | 57 |
| 14 | 0 | 40 |
| 15 | 0 | 83 |
| 16 | 0 | 77 |
| 17 | 0 | 12 |

[a] Counts begin at base of stem. Leaf 21 was 2.5 cm. long, leaf 17 was 28 cm. long, and had not quite reached its full size.

The results of similar tests on leaves of T.I. 448A infected with Valleau's "white"-mosaic virus are cited later.

## Observations Relating to Typical Mosaic Mottling in Wisconsin-Havana Seed

The results obtained with mutant *BSY* in T.I. 448A suggested a series of observations on mosaic-mottled leaves of Wisconsin-Havana Seed tobacco infected with *BSY* and supplementing those reported in 1929 (8). At that time the writer called attention to and illustrated the unusual symptoms that develop in the leaves of Wisconsin-Havana Seed tobacco when *BSY* virus invades well-developed, mature leaves. Reference to figure 8 in that paper (8) will show the characteristic "oak-leaf" pattern. These early observations made it clearly evident that the types of chlorotic symptoms induced in leaves are determined to a large extent by the stage of development of the leaf at the time of invasion.

Leaves of Wisconsin-Havana Seed tobacco with typical yellow-mosaic mottling were observed from the time they were 10 to 15 cm. long until they were mature. Normal green zones were carefully observed and it was

found that many of them remained normal green only a short time. In many cases yellow spots appeared, which resembled the secondary yellow spots that occurred in T.I. 448A tobacco.

Microscopic studies were carried out on small pieces of the various types of spotted, and mottled tissues and the normal green tissues cut from infected leaves of Wisconsin-Havana Seed tobacco. These pieces of tissue were dropped into absolute alcohol for a few minutes, then transferred to xylol for a like period. Partly cleared, they were then mounted in xylol and viewed through a compound microscope. With a magnification of 100×, it was possible to see through the epidermal cells and observe and count the palisade cells in both green and yellow areas.

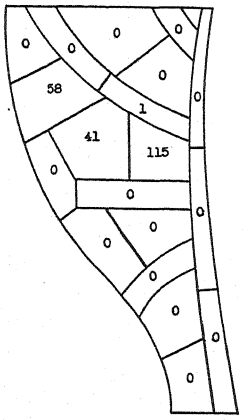



FIG. 6. Diagram of part of leaf from a plant of T.I. 448A infected with Nicotiana virus 1; no manifestation of symptoms on leaf; all sections outlined were assayed separately for virus; numerals denote number of lesions per bean plant.

These observations showed that (1) mottled leaves frequently contain normal green patches in which no yellow cells are detectable under the compound microscope; (2) patches that at first are normal green to the eye, in many cases develop a light or faded green; (3) these faded areas consist of small islands of palisade cells containing chlorophyll surrounded by yellow palisade cells; (4) the small green islands range from 1 to 20 or more palisade cells; (5) the faded green areas frequently, but not always, become completely yellow; and (6) this secondary yellowing in the normal green patches sometimes involves all palisade cells from the beginning of the process, resembling the progress of the secondary yellowing in perennial pepper leaves that have the local lesions of mutant *BSY* (14).

It seems reasonably evident from the observations cited that the occurrence of the typical mosaic patterns in foliage is dependent on virus entering the very small leaves, and that the mottling is modified by secondary chlorosis as the leaf develops.

## Inoculations with Miscellaneous Field Varieties of Nicotiana Virus 1 and Other Viruses

*"White"-mosaic virus.* This virus was supplied by W. D. Valleau. It is very similar to, but not identical with mutant *BSY*. In dark tobacco it induced severe yellow mosaic and was almost indistinguishable from *BSY* mosaic. In *Nicotiana glauca* it was isolated from top leaves in 80 per cent of the mature plants; it induced stem mottling and chlorotic spots in some of the leaves throughout the life of these plants. Mutant *BSY* was not completely systemic in any of the plants, and induced very few or no secondary chlorotic spots (9).

Mutant *BSY* and "white"-mosaic virus were each tested against Nicotiana virus 1 in Wisconsin-Havana Seed tobacco to determine the relative interference[6] or antagonism. The methods applied were the same as those reported for use with systemic viruses (12). It was found that Nicotiana virus 1 supplanted both of the other viruses, and that *BSY* virus was supplanted more quickly than the "white"-mosaic virus.

In two tests with T.I. 448A, the "white"-mosaic virus induced secondary light-green and yellow spots in 8 to 10 leaves per plant, with a maximum of 250 spots per leaf, whereas *BSY* virus induced similar spots on 4 to 5 leaves per plant, with a maximum of 25 spots per leaf.

Three leaves, each from a separate plant of T.I. 448A, were chosen for study. Leaf 1 had 22, leaf 2 had 77, and leaf 3 had 271 systemic yellow spots ("white" mosaic). There was enough normal-green tissue in each leaf to provide samples well removed from yellow spots. Tissue samples consisted of round discs 19 mm. in diameter. One disc was removed from an area containing 10 yellow spots; 12 discs, 4 from each leaf, were taken from normal-green zones flanked by scattered yellow spots at distances ranging from 5 to 20 mm. from the margins of the discs. Each disc was pulped in 3 cc. of buffered solution, and the resulting fluid was wiped on the two primary leaves of 3 bean plants.

The 4 normal-green discs from leaf 1 induced 0, 0, 1, and 1 lesions, respectively, on the 6 bean leaves; those from leaf 2 induced 800, 700, 300, and 1 lesions, respectively, per bean plant; and those from leaf 3 induced 90, 35, 0, and 0 lesions, respectively, per bean plant. The disc from the spotted tissue induced 1400 lesions per bean plant. The high counts were based on close estimates.

Fourteen days following the above assays, a few light-green and yellow spots appeared in some of the areas that were normal green at the time the sample discs were removed. It is not surprising, therefore, that virus was isolated from some of the normal-green tissue.

[6] In recent years it has been found that certain viruses attacking the mammals, exhibit antagonism. The phenomenon occurs between certain yellow-fever viruses and the Rift-Valley-fever virus (3), between some of the viruses of the poliomyelitis group (6), and between the virus of canine distemper and experimental poliomyelitis virus (2). Some virologists have regarded the phenomenon as a form of immunity in mammals, but the present trend is to refer to it as a "sparing effect" (2) and "interference" (3, 6), and to regard the phenomenon as being quite distinct from acquired immunity. This position is in line with the position taken by the writer (11) regarding the phenomenon in plants.

In another test with T.I. 448A, "white"-mosaic virus induced yellow spots in 4 consecutive leaves above the inoculated leaf. In the next 4 consecutive leaves, faintly chlorotic spots appeared that were detectable only in transmitted light, whereas, no signs of disease appeared in any of the well-developed leaves above nor in the tip leaves. Nondiluted extracts from each of these symptom-free leaves and from the tip cluster of small leaves were assayed on bean leaves. Proceeding in order up the plant, these extracts induced local lesions at the rate of 800, 30, 400, 2, 0, and 0 lesions per bean plant.

Trials with tobacco genotypes that carry the necrotic-lesion factor and trials with *Nicotiana sylvestris* Spegaz. and Comes, have shown these types to be inferior test plants for detecting traces of necrotic-lesion-inducing viruses. *Phaseolus vulgaris* L., when grown near 33.3° C. after inoculation, and *Nicotiana glutinosa* L., appear to be among the best known test plants for determining traces of the viruses under discussion. The fact that Valleau and Diachun (19) used a tobacco for their testing suggests one possible explanation for their failure to detect traces of virus in their normal-green tissue.

"White"-mosaic virus, like *BSY* virus, was not isolated from the growing tips of the stems and small leaves of infected plants of T.I. 448A. A typical test is cited. The tissue extracts were diluted in 5 parts of buffered solution and wiped on bean leaves dusted with carborundum powder. The extract from the growing points and smallest leaves and the extract from leaves 2.5 to 5 mm. long induced no lesions, whereas the extract from leaves 70 to 90 mm. long induced 350 lesions per bean plant. There were no chlorotic spots discernible on any of the tobacco leaves tested.

*Type B Yellow-mosaic Virus.* This virus was isolated from *Nicotiana glauca* L., growing in the Canary Islands (8). It is very similar to mutant *BSY,* but, unlike *BSY,* it induces severe yellow mosaic in *Nicotiana glauca* (9). In T.I. 448A tobacco, however, the two viruses induce symptoms that are practically indistinguishable.

*Plantago-mosaic Virus.* This virus was collected in 1941 on *Plantago major* L. at Arlington Farm, Virginia. On this species the disease is distinctly a ring spot. It was found in 3 separate locations, 2 of which were within a few feet of the entrances to 2 separate greenhouses; the other was close to a laboratory entrance. Common mosaic of tobacco had long been studied in these greenhouses and in the laboratory. Search for this disease on *P. major* and *P. lanceolata* L. in nearby Virginia, Maryland, and the District of Columbia has met with failure. Experiments have shown that infected leaves of *P. major* frequently show no signs of disease. This virus may be a strain of *Nicotiana virus* 1; it is similar to the virus collected by Holmes (5) on *P. major* and *P. lanceolata,* but, unlike his virus, it rarely induces local lesions, ring spots, or oak-leaf patterns on Turkish tobacco, var. Samsun.

In tobacco T.I. 448A, the virus induced no symptoms. Systemic virus was recovered from only one of the 30 inoculated plants, and this plant was one of a series of five inoculated by the carborundum-wipe method. The tests conducted thus far indicate that T.I. 448A tobacco manifests an unusually high degree of resistance against this virus.

*Nicotiana Virus 6.* This virus induced no local or secondary symptoms in T.I. 448A and only occasional, faint, light-green spots in Ambalema. In both genotypes, the virus seems to synthesize and progress much like Nicotiana virus 1.

*Type C Yellow-mosaic Virus.* This virus and Nicotiana virus 6 seem to belong to a closely related group occurring in *Nicotiana glauca* growing in the Canary Islands (9). In T.I. 448A no local or secondary symptoms occurred, and the virus made very slight progress in the few plants that have been studied.

*Solanum Virus 2.* Virus from veinbanding mosaic and virus from the "Y" mosaic induced typical symptoms in T.I. 448A. This tobacco is regarded as highly susceptible to both of these mosaics.

*Cucumis Virus 1.* Virus from the milkweed-cucumber source induced mosaic without necrosis in T.I. 448A; but virus from the celery-mosaic source induced local chlorotic spots and compound necrotic lesions and systemic necrosis in stems and young leaves.

*Nicotiana Virus 12* (tobacco ring spot). T.I. 448A is very susceptible to the acute symptoms, and manifests the low-temperature chlorosis and mosaic mottling associated with the chronic phase (11) of this disease.

## DISCUSSION AND CONCLUSIONS

When viruses of the yellow-mosaic mutants were used to inoculate tobacco T.I. 448A, the secondary chlorotic spots rarely appeared in the new leaves until they had attained about $\frac{1}{4}$ to $\frac{1}{2}$ of their growth. Traces of virus, however, were detected occasionally in tip leaves 5 to 6 cm. long. The virus either does not enter or increase perceptibly in the very young leaves, which probably explains the absence of true mosaic mottling in T.I. 448A. The first signs of secondary chlorotic spots usually appear at or near the tip of the leaf. Spotting tends to progress down the leaf as it grows. The spread of chlorosis from the initial spots tends to be greatest in the leaf margins and tip. Thus it appears that this spread is not correlated with the rate of cell division, as division is less rapid toward the tip than it is in the lower margins (1).

The evidence available makes it rather conclusive that the secondary chlorotic spots in T.I. 448A and Ambalema result from the destruction of chlorophyll rather than from the prevention of chlorophyll synthesis. Products of a deranged metabolism may be the direct cause of the destruction of chlorophyll in these tobaccos, but good circumstantial evidence of this cannot be obtained so readily as in perennial pepper, *Capsicum frutescens* L. (14).

In the highly susceptible genotypes, such as Wisconsin-Havana Seed tobacco, it appears that the amount of virus in the very small leaves, although less than in leaves at a more advanced stage, is sufficient to induce the necessary reactions that prevent or reduce the synthesis of chlorophyll in certain groups of parenchyma cells. It may be that these cell groups are more susceptible than those in which the normal quota of chlorophyll is produced. However, from the observations cited for T.I. 448A and Wisconsin-Havana Seed tobaccos, it appears more likely that the initial quantity of virus entering the youngest leaves in a highly susceptible tobacco may be too small to insure all parenchyma cells receiving their quota of virus before the chlorophyll is synthesized, and that those cells receiving virus after the appearance of chlorophyll do not become chlorotic for a time at least, depending on the virus used. Thus, the classical mosaic pattern results.

With common mosaic (Nicotiana virus 1), the classical mosaic pattern in dark-green tobaccos tends to change very little as the leaves develop, whereas, with yellow mosaics, the mosaic pattern tends to be less permanent, becoming modified because of the advance of delayed chlorosis into the green zones as the leaves develop.

The evidence against the free movement of the common-mosaic virus and the yellowing strains in T.I. 448A tobacco, and the evidence for delayed movement of common-mosaic virus out of the initial infection sites in highly susceptible tobacco (4) and tomato (7), suggests that relatively few virus particles move long distances in the plant (13). It is to be expected that molecules or micellae of the immense size of the virus will not move about in the cytoplasm (15) and through the plasmodesmata and other channels as freely as sugars and other solutes.

It is of special interest that none of the mutants from the common-mosaic virus seemed to have greater invasive powers than the common-mosaic virus, and some of them seemed less invasive. None of these strains nor Nicotiana virus 1 induces local or systemic necrosis in T.I. 448A. Very small local necrotic lesions occurred on the leaves of Ambalema tobacco, but no systemic necrosis has been observed.

The strain of *Nicotiana glauca* used by the writer (8, 9) is variously similar to T.I. 448A and Ambalema tobaccos with respect to the resistance to the common-mosaic virus and yellow strains used in these studies. However, when a series of strains is tested on T.I. 448A and *N. glauca,* the exact order of virulence obtaining in one host is not reflected in the other. The results obtained with virus strains *BSY,* type *B* and "white" mosaic illustrate this point. Furthermore, the collection of *N. glauca* used does not react in the same way to different collections of the light-green-mosaic viruses that clearly belong in the common-mosaic group (8, 9). Such results make it rather clear that a single strain of virus cannot be used for indicating the precise mosaic-resistance-gene make-up in mosaic-resistant plants. Certain yellow-mosaic strains may serve as rough indicators of resistance to the wild or field-type of common mosaic virus, but the complete genetic picture cannot be obtained in this manner.

In the genus *Nicotiana*, the various gene complexes regulating symptom expressions induced by a given virus strain seem to show as much diversity as is shown among the various strains of the common-mosaic virus when compared on a given suitable genotype. In breeding work, these gene complexes seem to combine in so many different ways as to make it appear highly probable that all the strains of the common-mosaic virus would have to be used as testers in order to detect all of the gene complexes controlling the various types and degrees of disease expression in the genus.

Plants of T.I. 448A and Ambalema tobacco infected with Nicotiana virus 1 have never shown yellow-mosaic mutation spots (9) in the writer's tests, either in the field or in the greenhouse. Since all of the evidence available indicates that Nicotiana virus 1 interferes with the unlimited increase and spread of its mutants (9, 12), it seems highly probable that these resistant tobaccos may be regarded as very poor natural reservoirs for mutant viruses arising from Nicotiana virus 1. If at some future time it is discovered that Nicotiana 1 does give rise to mutants that suppress it, the problem will be changed and difficulties may be encountered with all of the types of resistant tobacco now in use.

Tobacco T.I. 448A appears to be one of the best genotypes, if not the best, now available for use in breeding for resistance against the common-mosaic virus and its mutants. Some of the genotypes isolated from the crosses studied by Nolla (16) and by Valleau (19) may be just as resistant as T.I. 448A, but this point can be settled only from the results of comparative tests, as the published results leave too many points in doubt.

BUREAU OF PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND.

LITERATURE CITED

1. AVERY, GEORGE S., JR. Structure and development of the tobacco leaf. Amer. Jour. Bot. 20: 565–592. 1933.
2. DALDORF, GILBERT, MARGARET DOUGLASS, and H. E. ROBINSON. The sparing effect of canine distemper on poliomyelitis in *Macaca mulatta*. Jour. Exp. Med. 67: 333–343. 1938.
3. FINDLAY, G. M., and F. O. MACCALLUM. An interference phenomenon in relation to yellow fever and other viruses. Jour. Path. and Bact. 44: 405–424. 1937.
4. HOLMES, FRANCIS O. Local and systemic increase of tobacco-mosaic virus. Amer. Jour. Bot. 17: 789–805. 1930.
5. ————. A distinctive strain of tobacco-mosaic virus from *Plantago*. Phytopath. 31: 1089–1098. 1941.
6. JUNGEBLUT, CLAUS W., and MURRAY SANDERS. Studies of a murine strain of poliomyelitis virus in cotton rats and white mice. Jour. Exp. Med. 72: 407–436. 1940.
7. KUNKEL, L. O. Movement of tobacco-mosaic virus in tomato plants. Phytopath. 29: 684–699. 1939.
8. MCKINNEY, H. H. Mosaic diseases in the Canary Islands, West Africa and Gibraltar. Jour. Agr. Res. [U. S.] 39: 557–558. 1929.
9. ————. Evidence of virus mutation in the common mosaic of tobacco. Jour. Agr. Res. [U. S.] 51: 951–981. 1935.
10. ————. Comparative studies on two genotypes of *Nicotiana tabacum* resistant to Nicotiana virus 1. Phytopath. 29: 16. 1939.
11. ————. Virus antagonism, natural host resistance and the acquired-immunity concept. Phytopath. 31: 1059–1061. 1941.

12. ————————. Virus antagonism tests and their limitations for establishing relationship between mutants, and nonrelationship between distinct viruses. Amer. Jour. Bot. 28: 770–778. 1941.
13. ————————. Studies on genotypes of tobacco resistant to the common-mosaic virus. Phytopath. 33: 300–313. 1943.
14. ————————, and CLAUDE H. HILLS. Mosaic, chlorosis and necrosis in virus-infected perennial pepper caused directly by products of a deranged metabolism. Science 94: 372–373. 1941.
15. MARTIN, LAWRENCE F., and H. H. MCKINNEY. Tobacco-mosaic virus concentrated in the cytoplasm. Science 88: 458–459. 1938.
16. NOLLA, J. A. B. Inheritance in *Nicotiana*. III. A study of the character of mosaic resistance in *Nicotiana tabacum* L. Jour. Hered. 29: 43–48. 1938.
17. PETERSON, PAUL D. The influence of four mosaic diseases on the plastid pigments and chlorophyllase in tobacco leaves. Phytopath. 28: 329–342. 1938.
18. SMITH, KENNETH M. A text book of plant virus diseases. 615 pp., 101 fig. P. Blakiston's Son and Co. (Philadelphia). 1937.
19. VALLEAU, W. D., and STEPHEN DIACHUN. Virus distribution in mosaic-resistant tobacco and its relation to pattern development in susceptible varieties. Jour. Agr. Res. [U. S.] 62: 241–248. 1941.

# A STUDY OF THE GENETICS OF SOROSPORIUM SYNTHERISMAE AND SPHACELOTHECA PANICI-MILIACEI[1]

W. J. MARTIN

(Accepted for publication October 1, 1942)

It is important for the plant breeder to know the range in variation in the fungi that cause plant diseases in order that he may work most efficiently in the production of improved varieties. A better knowledge of the extent and nature of variation in the smut fungi that attack grasses, therefore, should be beneficial to the plant breeder in the improvement of grasses.

There are over 100 species of smut fungi that attack approximately 250 species of grasses in the United States (1), but very little is known about variation in many of these species. Davis (5), Fischer (6, 7), and others (2, 11) have contributed greatly to our knowledge of some species that attack grasses. Martin and Kernkamp (15) found numerous biotypes in each of 5 species from grasses and reported the production of variants in monosporidial cultures of these smuts. Subsequently, *Sorosporium syntherismae* (Pk.) Farl. and *Sphacelotheca panici-miliacei* (Pers.) Bubak were studied more thoroughly to determine the extent and nature of variation resulting from mutation and hybridization. The results are given in this paper.

*Sorosporium syntherismae* is a head smut that has been reported on the following hosts: *Panicum agrostoides* Spreng., *P. capillare* L., *P. dichotomiflorum* Michx., *P. hirticaule* Presl., *P. stramineum* Hitchc. and Chase, *Cenchrus echinatus* L., *C. incertus* M. A. Curtis, *C. pauciflorus* Benth., and *C. tribuloides* L. *Sphacelotheca panici-miliacei,* also a head smut, occurs on *Panicum miliaceum* only (1, 24).

The literature on the genetics of the smut fungi has been summarized by Christensen and Rodenhiser (3) and by Miss Sampson (18); therefore, no general review of the literature is given, but pertinent literature is discussed in the text when deemed necessary.

## MATERIALS AND METHODS

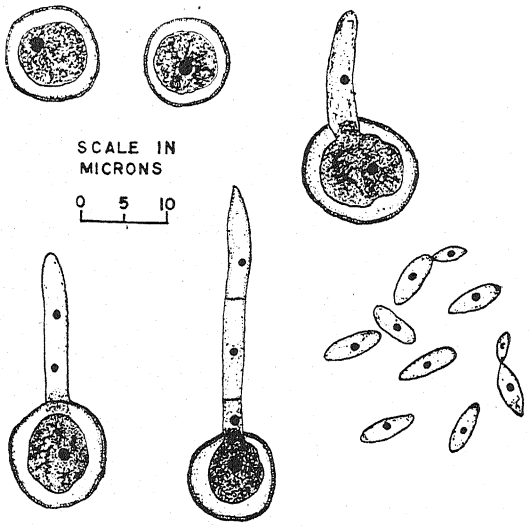
The collections of *Sorosporium syntherismae* were obtained from *Panicum capillare* and *Cenchrus pauciflorus,* two common hosts of that species; the collection of *Sphacelotheca panici-miliacei* came from *Panicum miliaceum.* The collections of the former conformed generally to Clinton's description of that species (4), while that of the latter agreed with Zundel's description (24).

[1] Summary of a thesis presented in partial fulfillment of the requirements for the degree Doctor of Philosophy, granted by the University of Minnesota, June, 1942.

Paper No. 2030 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

Assistance in the preparation of these materials was furnished by the personnel of the Works Progress Administration, Official Project No. 165-71-1-124. Sponsored by the University of Minnesota, 1942.

The seeds of grasses used in the experiments were obtained from the following sources: *Panicum miliaceum* from A. C. Arny of the Division of Agronomy and Plant Genetics, University of Minnesota; *Panicum capillare* and *Cenchrus pauciflorus* from field collections made by the writer in 1940 at University Farm, St. Paul, Minnesota. All the seeds were treated in a



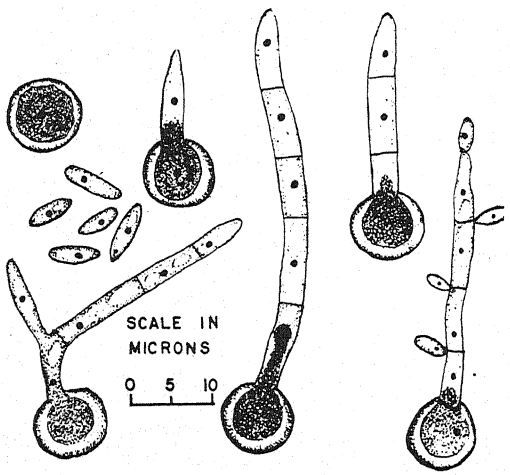




FIG. 1. Camera-lucida drawings of germinating chlamydospores of *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* stained with iron-alum haematoxylin.

1:250 solution of formaldehyde for 10 minutes, washed for 4 hours in running water, and then allowed to dry.

For inoculation purposes, monosporidial lines were grown in potato-dextrose broth containing 15 per cent dextrose; the high sugar content in-

duced a sporidial rather than a mycelial type of growth (13), which is highly desirable, especially when inoculations are made with a hypodermic syringe. Inoculations were made by injecting 10-day-old cultures into young seedlings, or else by soaking the seeds in the broth cultures for 24 hours shortly before planting.

## EXPERIMENTAL RESULTS

### Germination and Cytology of Chlamydospores

Chlamydospores of both *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* usually germinated by producing septate promycelia with lateral sporidia; however, in both species certain promycelial cells commonly produced hyphal branches instead of sporidia. Four-celled promycelia generally were uncommon in both species. The type of germination varied

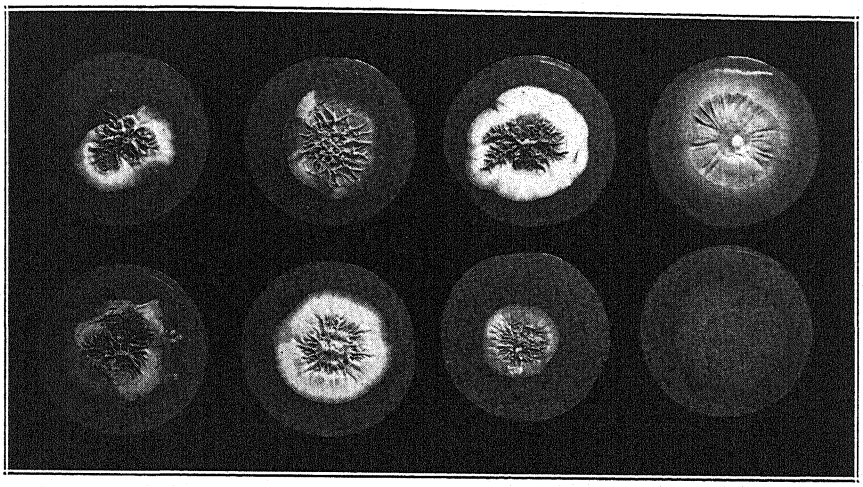


FIG. 2. Monosporidial cultures of *Sphacelotheca panici-miliacei* on potato-dextrose agar, showing a few cultural races. Note sectoring in the two cultures at left.

greatly among chlamydospores of the same collection, as described recently by Kernkamp and Petty (14) for *Ustilago zeae*. No distinguishing differences in the type of germination were observed between chlamydospores of *S. syntherismae* from *Panicum* and those from *Cenchrus*, although Norton (16) observed such differences in the collections with which he worked.

Harper's iron-alum haematoxylin technique was used (8) for studying the nuclear condition in the chlamydospores and sporidia. The chlamydospores, promycelial cells, and sporidia of both species were uninucleate (Fig. 1). Attempts to find meiotic figures were unsuccessful, but, since the writer observed that the very young chlamydospores are binucleate, and that infection occurs only when certain combinations of monosporidial lines are used in the inoculations, he assumed that the nuclear condition in these two smut fungi is similar to that in others, as reported by Wang (22) and others (see 3). That is, the sporidia normally are haploid and potentially gametic,

a dicaryotic condition is initiated at the time of infection, the diploid nucleus is formed at the time of maturation of the chlamydospore, and reduction division occurs when the chlamydospore germinates. The fusion of sporidia of opposite sex under cultural conditions, which is common in many of the smut fungi (see 3), was not observed with any degree of certainty, although repeated experiments over a wide range of conditions were made. However, sporidial fusions in *Ustilago zeae* have been seen but rarely, also (20).

## Cultural Races, Mutation, and Intraspecific Hybridization

Approximately 150 monosporidial cultures of *Sorosporium syntherismae* from *Panicum capillare*, 130 of *S. syntherismae* from *Cenchrus pauciflorus*, and 120 of *Sphacelotheca panici-miliacei* from *Panicum miliaceum* were isolated. All of these isolates were grown on potato-dextrose agar and on two per cent malt agar in 250-cc. Erlenmeyer flasks. There were many cultural races in each species, and sectors frequently appeared in monosporidial isolates, as illustrated in figure 2. Such sector variants retained their distinctive characteristics when transferred to other flasks of agar, and could be maintained thus. Sectors often appeared in cultures that had originated from a mutant. Johnson, Rodenhiser, and Lefebvre (11) recorded a mutation in *S. syntherismae*, in which the mutant appeared in the form of buff sori on fall *Panicum*.

A comparison of the cultural characters of monosporidial isolates of *Sorosporium syntherismae* from *Cenchrus pauciflorus* with isolates of the same species from *Panicum capillare* showed that the isolates from certain collections from *C. pauciflorus* have a tendency to be somewhat different from those obtained from *P. capillare*, although this was not true for all collections.

Cultures obtained from single sporidia isolated from chlamydospores produced by crossing two monosporidial lines that originated from the promycelium of the same chlamydospore, differed strikingly in their cultural characters on potato-dextrose agar. Furthermore, none of the $f_1$ sporidial lines[2] was identical in cultural characters with either of the parental monosporidial lines, although some of the $f_1$ lines resembled one or the other parental line. That very few of the $f_1$ sporidial lines are identical with either of the parental lines also is true in the case of *Ustilago zeae* (20). Thus it appears that there are several factors governing cultural characters, and that there is a great deal of recombination of these factors, with segregation at the time of reduction division.

## The Relative Pathogenicity of the Two Species

Cross inoculations were made with collections of *Sorosporium syntherismae* from *Cenchrus pauciflorus* and *Panicum capillare* and with *Sphacelotheca panici-miliacei* from *Panicum miliaceum*. Inoculations were made by soaking the seeds in a heavy chlamydospore suspension in water

[2] The term "$f_1$ sporidial line" is used in designating the monosporidial lines obtained from the $F_1$ chlamydospores; the small f is used since they are gametic lines.

for 24 hours, then the seeds were filtered off, allowed to dry, and planted. The results of these inoculations are summarized in table 1.

TABLE 1.—*Percentage of smut*[a] *produced on three species of grasses as a result of artificial inoculation with Sorosporium syntherismae and Sphacelotheca panici-miliacei*

| Species of grass inoculated | Source of inoculum used and per cent smutted plants | | |
|---|---|---|---|
| | *S. syntherismae* from *Cenchrus pauciflorus* | *S. syntherismae* from *Panicum capillare* | *S. panici-miliacei* from *Panicum miliaceum* |
| *Cenchrus pauciflorus* ... | 1.8 | 1.2 | 0.0 |
| *Panicum capillare* ..... | 6.3 | 8.7 | 0.0 |
| *Panicum miliaceum* ..... | 16.8 | 12.6 | 65.7 |

[a] Percentage based on about 200 plants.

The percentage infection with *S. syntherismae* from either *Cenchrus* or *P. capillare* was very low in all the trials, particularly on *Cenchrus*. Attempts to obtain better infection by controlling various environmental factors, such as temperature and moisture, were unsuccessful. Johnson, Rodenhiser, and Lefebvre (11) reported good infection with *S. syntherismae* on *Panicum dichotomiflorum* Michx., but their methods were ineffective when tried with the two hosts used in this study.

Thus, *Sorosporium syntherismae* from either *Cenchrus pauciflorus* or *Panicum capillare* infected both of these species, and also *Panicum miliaceum;* while *Sphacelotheca panici-miliacei* infected only *P. miliaceum.* That *S. syntherismae* attacks *P. miliaceum* had not been clearly demonstrated previously.

Inoculations with monosporidial lines and with paired combinations of monosporidial lines of both *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* demonstrated that only certain paired combinations were capable of causing infection;[3] inoculations with other paired combinations and with individual monosporidial lines failed to cause infection. That paired combinations of opposite sex will cause infection, while paired com-

TABLE 2.—*Results of inoculating Panicum miliaceum with all possible combinations of eight monosporidial lines of Sorosporium syntherismae*

| 1 | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $E_1$ | $E_2$ | $E_3$ | $E_4$ |
|---|---|---|---|---|---|---|---|---|
| $D_1$ | − | − | + | − | − | − | − | + |
| $D_2$ | − | − | − | + | + | − | − | − |
| $D_3$ | + | − | − | + | + | − | − | − |
| $D_4$ | − | + | + | − | − | − | + | + |
| $E_1$ | − | + | + | − | − | − | − | + |
| $E_2$ | − | − | − | − | − | − | + | − |
| $E_3$ | − | − | − | + | − | + | − | − |
| $E_4$ | + | − | − | + | + | − | − | − |

+ = caused infection.
− = did not cause infection.

[3] The criterion of infection as used here was the production of chlamydospores in the inflorescence or part of the inflorescence.

binations of the same sex will not, is in accordance with the behavior of other smut species that have been studied (3).

Results of inoculations with 8 monosporidial lines of *Sorosporium syntherismae* in all possible combinations showed the presence of 7 distinct "compatibility groups" in these 8 lines (Table 2). With the exception of lines $1D_3$ and $1E_4$ (alike in their ability to combine with other lines) each line differs in ability to combine with the other lines. Likewise, the results of inoculations with 4 monosporidial lines of *Sphacelotheca panici-miliacei* in all possible combinations demonstrated the presence of four compatibility groups in these four lines (Table 3). The occurrence of more than two compatibility groups in the smut fungi has been reported by several investigators (2, 3) and such groups have been referred to as sex groups.

TABLE 3.—*Results of inoculating Panicum miliaceum with all possible combinations of four monosporidial lines of Sphacelotheca panici-miliacei*

| 10C | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | − | − | + | − |
| 2 | − | − | − | + |
| 3 | + | − | − | + |
| 4 | − | + | + | − |

+ = caused infection.
− = did not cause infection.

## Interspecific Hybridization

Reports in the literature of the presence in nature of forms intermediate between *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* suggested the possibility of the occurrence of hybridization between the two species. Zundel (24) states in a note below his description of *S. panici-miliacei* that certain collections on *Panicum miliaceum* closely resemble *S. syntherismae*. He states that these specimens have "larger, more irregular spores and, under an immersion-lens, often show more or less evident indications of verruculations in their walls." According to Winter (23), Fischer von Waldheim also observed such specimens and placed them in what is now called *S. panici-miliacei*. Winter states: "Die Sporen sind an allen von mir untersuchten Exemplaren glatt, während sie nach Fischer von Waldheim stachelig sein sollen." Certain of these specimens may have been *S. syntherismae* as the present writer showed that *P. miliaceum* can become infected by this species.

To test the possibility of interspecific hybridization between *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei*, experiments were made in which a monosporidial line of one species was mixed with a monosporidial line of the other species, and the mixture injected into young grass seedlings by means of a hypodermic syringe. Seedlings of *Panicum miliaceum*, a host common to both species, and seedlings of *Panicum capillare*, a host of *S. syntherismae*, were inoculated with the interspecific combinations. As controls, seedlings were injected with each monosporidial line that entered

the combinations (Table 4). Certain of the interspecific combinations caused infection with the production of chlamydospores, while other combinations and the single monosporidial lines did not. This indicated that hybridization had occurred between the two species.

Inoculations with interspecific combinations then were made in which seeds of the White Ural variety of *Panicum miliaceum* were soaked in the various monosporidial combinations given in table 5. Each paired combination and each monosporidial line entering the combinations was used in inoculating lots of 175 seeds, each of which was then planted in two 9-foot rows in the field. The results of these inoculations are given in table 5.

The results of the inoculations in the field were similar to those in the greenhouse in that certain of the interspecific combinations caused infection,

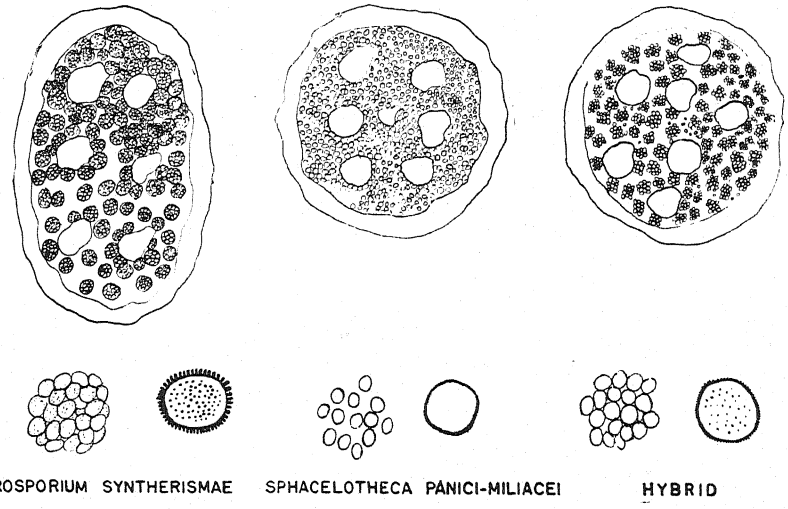



FIG. 3. Sori in cross section and spores of *Sorosporium syntherismae, Sphacelotheca panici-miliacei,* and the interspecific hybrid on *Panicum miliaceum.*

while other combinations and single monosporidial lines did not. Twenty-three of the 47 interspecific combinations used in inoculating plants of *Panicum miliaceum* were compatible and produced mature chlamydospores. Four of the 15 different interspecific combinations used in inoculating plants of *Panicum capillare* produced mature chlamydospores on that host. This indicated that interspecific hybridization had occurred, and to confirm this, a study was made of the $F_1$ chlamydospores and their progeny. The $F_1$ chlamydospores from certain of the crosses were used in inoculating *P. miliaceum* to obtain $F_2$ chlamydospores and certain of these in turn were used in inoculating plants to obtain $F_3$ chlamydospores. The inheritance of several characters was studied.

## Inheritance of Characters in the Interspecific Hybrid

*Wall Markings of Chlamydospores.* Numerous intraspecific crosses (crosses between monosporidial lines of the same species) within both *Soro-*

*sporium syntherismae* and *Sphacelotheca panici-miliacei* were made, and in all cases those crosses within *S. syntherismae* resulted in the production of verruculose chlamydospores and those within *S. panici-miliacei* resulted in smooth chlamydospores. In all the interspecific crosses, the $F_1$ chlamydo-

TABLE 4.—*The results of inoculating plants of Panicum miliaceum and Panicum capillare with interspecific sporidial combinations of Sorosporium syntherismae × Spacelotheca panici-miliacei in the greenhouse*

| Combinations *S. syn.* × *S. p–m* | *Panicum miliaceum* | | | *Panicum capillare* | | |
|---|---|---|---|---|---|---|
| | Number of plants | | Percentage smutted plants | Number of plants | | Percentage smutted plants |
| | Inoculated | Smutted | | Inoculated | Smutted | |
| $J_1 \times 5A_2$ | 32 | 0 | 0.0 | 35 | 0 | 0.0 |
| $J_2 \times$ " | 105 | 39 | 37.1 | 30 | 4 | 13.3 |
| $J_3 \times$ " | 42 | 0 | 0.0 | 36 | 0 | 0.0 |
| $J_4 \times$ " | 87 | 36 | 41.4 | 37 | 6 | 16.2 |
| $J_5 \times$ " | 45 | 12 | 26.7 | 30 | 3 | 10.0 |
| $J_1 \times 5C_2$ | 26 | 0 | 0.0 | 18 | 0 | 0.0 |
| $J_2 \times$ " | 22 | 0 | 0.0 | 15 | 0 | 0.0 |
| $J_3 \times$ " | 28 | 0 | 0.0 | 14 | 0 | 0.0 |
| $J_4 \times$ " | 31 | 9 | 29.0 | 20 | 0 | 0.0 |
| $J_5 \times$ " | 29 | 8 | 27.6 | 20 | 0 | 0.0 |
| $J_1 \times 5D_1$ | 21 | 0 | 0.0 | 16 | 0 | 0.0 |
| $J_2 \times$ " | 20 | 9 | 45.0 | 15 | 2 | 13.3 |
| $J_3 \times$ " | 25 | 0 | 0.0 | 19 | 0 | 0.0 |
| $J_4 \times$ " | 28 | 7 | 25.0 | 21 | 0 | 0.0 |
| $J_5 \times$ " | 26 | 8 | 30.8 | 22 | 0 | 0.0 |
| $J_1 \times 10C_1$ | 29 | 10 | 34.5 | ..... | ..... | ..... |
| " $\times 10C_2$ | 18 | 0 | 0.0 | ..... | ..... | ..... |
| " $\times 10C_3$ | 22 | 3 | 13.6 | ..... | ..... | ..... |
| " $\times 10C_4$ | 26 | 1 | 3.9 | ..... | ..... | ..... |
| $J_2 \times 10C_1$ | 22 | 12 | 54.5 | ..... | ..... | ..... |
| " $\times 10C_2$ | 20 | 6 | 30.0 | ..... | ..... | ..... |
| " $\times 10C_3$ | 25 | 4 | 16.0 | ..... | ..... | ..... |
| " $\times 10C_4$ | 28 | 0 | 0.0 | ..... | ..... | ..... |
| $J_1$ Ck. | 70 | 0 | 0.0 | 36 | 0 | 0.0 |
| $J_2$ Ck. | 77 | 0 | 0.0 | 30 | 0 | 0.0 |
| $J_3$ Ck. | 58 | 0 | 0.0 | 35 | 0 | 0.0 |
| $J_4$ Ck. | 79 | 0 | 0.0 | 33 | 0 | 0.0 |
| $J_5$ Ck. | 54 | 0 | 0.0 | 36 | 0 | 0.0 |
| $5A_2$ Ck. | 54 | 0 | 0.0 | 31 | 0 | 0.0 |
| $5C_2$ Ck. | 24 | 0 | 0.0 | 18 | 0 | 0.0 |
| $5D_1$ Ck. | 26 | 0 | 0.0 | 22 | 0 | 0.0 |
| $10C_1$ Ck. | 18 | 0 | 0.0 | ..... | ..... | ..... |
| $10C_2$ Ck. | 16 | 0 | 0.0 | ..... | ..... | ..... |
| $10C_3$ Ck. | 18 | 0 | 0.0 | ..... | ..... | ..... |
| $10C_4$ Ck. | 22 | 0 | 0.0 | ..... | ..... | ..... |

spores were verruculose and somewhat similar to those of the *S. syntherismae* parent (Fig. 3).

Eighty-nine $F_2$ sori from 3 interspecific crosses were examined microscopically, and the character of the spore walls recorded (Table 6). The ratio of sori containing verruculose spores to those containing glabrous spores approaches 3 : 1, and thus it appears that the factor for verruculose spores behaves as a simple dominant in this interspecific cross. This is in

accordance with other reports of interspecific hybrids between species of smut fungi having smooth and rough walls; *i.e.*, the factor for roughness is dominant over that for smoothness (3, 9, 17).

TABLE 5.—*The results of inoculating plants of Panicum miliaceum with interspecific sporidial combinations of Sorosporium syntherismae × Sphacelotheca panici-miliacei in the field*

| Combinations *S. syn.* × *S. p–m* | Number of panicles | | |
|---|---|---|---|
| | Total | Smutted | Percentage smutted |
| $J_1 \times 10C_1$ | 278 | 0 | 0.0 |
| $J_2 \times$ " | 261 | 0 | 0.0 |
| $J_3 \times$ " | 212 | 21 | 9.9 |
| $J_4 \times$ " | 221 | 0 | 0.0 |
| $J_1 \times 10C_2$ | 203 | 0 | 0.0 |
| $J_2 \times$ " | 166 | 38 | 22.9 |
| $J_3 \times$ " | 205 | 0 | 0.0 |
| $J_4 \times$ " | 188 | 90 | 47.9 |
| $J_1 \times 10C_3$ | 196 | 30 | 15.3 |
| $J_2 \times$ " | 180 | 0 | 0.0 |
| $J_3 \times$ " | 195 | 0 | 0.0 |
| $J_4 \times$ " | 160 | 0 | 0.0 |
| $J_1 \times 10C_4$ | 151 | 13 | 8.6 |
| $J_2 \times$ " | 137 | 0 | 0.0 |
| $J_3 \times$ " | 186 | 22 | 11.8 |
| $J_4 \times$ " | 173 | 0 | 0.0 |
| $J_1 \times 10D_1$ | 145 | 0 | 0.0 |
| $J_2 \times$ " | 192 | 163 | 84.9 |
| $J_3 \times$ " | 145 | 0 | 0.0 |
| $J_4 \times$ " | 183 | 66 | 36.1 |
| $J_1 \times 10D_2$ | 228 | 0 | 0.0 |
| $J_2 \times$ " | 212 | 121 | 57.1 |
| $J_3 \times$ " | 241 | 0 | 0.0 |
| $J_4 \times$ " | 166 | 92 | 55.4 |
| $J_1 \times 10D_3$ | 206 | 0 | 0.0 |
| $J_2 \times$ " | 226 | 178 | 78.8 |
| $J_3 \times$ " | 235 | 0 | 0.0 |
| $J_4 \times$ " | 214 | 133 | 62.1 |
| $J_1 \times 10D_4$ | 198 | 0 | 0.0 |
| $J_2 \times$ " | 171 | 0 | 0.0 |
| $J_3 \times$ " | 192 | 0 | 0.0 |
| $J_4 \times$ " | 184 | 0 | 0.0 |
| $J_1$ Ck. | 210 | 0 | 0.0 |
| $J_2$ Ck. | 165 | 0 | 0.0 |
| $J_3$ Ck. | 197 | 0 | 0.0 |
| $J_4$ Ck. | 235 | 0 | 0.0 |
| $10C_1$ Ck. | 193 | 0 | 0.0 |
| $10C_2$ Ck. | 240 | 0 | 0.0 |
| $10C_3$ Ck. | 210 | 0 | 0.0 |
| $10C_4$ Ck. | 212 | 0 | 0.0 |
| $10D_1$ Ck. | 172 | 0 | 0.0 |
| $10D_2$ Ck. | 183 | 0 | 0.0 |
| $10D_3$ Ck. | 179 | 0 | 0.0 |
| $10D_4$ Ck. | 205 | 0 | 0.0 |

Inoculation of plants of *Panicum miliaceum* with smooth $F_2$ chlamydospores resulted in the production of sori that contained smooth spores; in-

TABLE 6.—*Segregation for spore wall markings in 89 $F_2$ sori from three interspecific crosses between Sorosporium syntherismae and Sphacelotheca panici-miliacei*

| Interspecific cross *S. syn.* × *S. p–m* | Number of sori | | |
|---|---|---|---|
| | Total | With smooth spore walls | With verruculose spore walls |
| $J_4 \times 10D_1$ | 23 | 5 | 18 |
| $J_4 \times 10C_2$ | 28 | 6 | 22 |
| $J_2 \times 10D_1$ | 38 | 9 | 29 |
| Total | 89 | 20 | 69 |

oculations with verruculose $F_2$ chlamydospores resulted in the production of some sori with verruculose spores and others with both verruculose and glabrous spores.

Attempts were made to mate the $f_1$ sporidial lines from the various interspecific courses with the parental monosporidial lines used in making the interspecific cross. Each of the 65 $f_1$ sporidial lines obtained was paired with each parental line, and plants of *Panicum miliaceum* were inoculated with combinations. Although repeated experiments were made, only 4 of these paired combinations caused infection. Two monosporidial lines from cross 22 (*Sorosporium syntherismae* $J_2 \times$ *Sphacelotheca panici-miliacei* $10D_1$) were compatible with the *S. panici-miliacei* parent, and two other lines were compatible with the *S. syntherismae* parent. The two crosses involving an $f_1$ sporidial line and a line from the Sorosporium parent resulted in the production of verruculose spores, as was expected; the two crosses with the *Sphacelotheca* parent yielded smooth spores. Numerous attempts were made to obtain a larger number of such crosses to determine whether the expected 1:1 ratio of verruculose to glabrous spores would result from crossing $f_1$ sporidial lines with the parental line from the *Sphacelotheca* parent. For some unknown reason, however, only 4 of the 130 paired combinations caused infection.

*Size of Chlamydospores.* The $F_1$ hybrid chlamydospores from interspecific crosses were intermediate in size as compared with chlamydospores from intraspecific crosses in *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei,* respectively, as is shown in table 7. The difference in spore size for the two species, however, is small, and attempts to follow this character in the $F_2$ generation were unsuccessful. Nevertheless, it was possible to find the larger, smooth spores and also the smaller verruculose spores in the $F_2$

TABLE 7.—*The size of chlamydospores*[a] *of Sorosporium syntherismae, Sphacelotheca panici-miliacei, and a hybrid between the two species*

| Smut species or hybrid | Range in microns | Average in microns |
|---|---|---|
| *Sorosporium syntherismae* | 8.4–12.0 × 7.0–10.9 | 9.8 × 8.5 |
| *Sphacelotheca panici-miliacei* | 7.0–10.2 × 6.0–10.1 | 8.2 × 7.8 |
| Hybrid (*S. syn* $J_4 \times$ *S. p–m.* $5A_2$) | 7.0–11.5 × 6.4–10.5 | 8.7 × 7.9 |

[a] Two hundred chlamydospores of each species and of the hybrid were measured.

generation of the interspecific crosses, while in the intraspecific crosses the smaller spores (those of *S. panici-miliacei*) are smooth and the larger spores (those of *S. syntherismae*) are verruculose. Thus it appears that the factors for wall markings and size of the chlamydospore are inherited independently in the interspecific hybrid, or, if they are linked, crossing-over occurs frequently.

*Germination of Chlamydospores and Viability of Sporidia.* The $F_1$ hybrid chlamydospores germinated by producing septate promycelia with lateral sporidia, as do chlamydospores of both parent species. There was, however, a difference among chlamydospores of both the smut species and the hybrid in the time required for germination. Two different interspecific hybrids were compared with the two parent species: the chlamydospores from the two interspecific hybrids required 10 and 11 hours for germination, whereas those of *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* required 20 hours and 7 hours, respectively.

Most of the interspecific hybrid chlamydospores germinated and produced primary sporidia, a high percentage of which, however, failed to bud. In both parent species over 90 per cent of the single sporidial isolates developed into microscopic colonies, whereas less than 10 per cent of over 700 single sporidial isolates from the $F_1$ hybrid chlamydospores developed into colonies. Many of the sporidia that were isolated gradually underwent lysis without budding; others budded a few times, then underwent lysis, while a few others budded and developed into colonies. Not a single complete set of monosporidial cultures from a hybrid chlamydospore was obtained, even though over 100 such complete sets were isolated. This low viability of $f_1$ sporidia from interspecific crosses in the smut fungi has been reported previously (9, 10), and it appears to be a type of hybrid sterility. Sporidia from the $F_2$ hybrid chlamydospores did not undergo lysis.

*Pathogenicity.* The interspecific dicaryophytes, *i.e.*, those dicaryophytes that arose from the union of a haploid line of one species with a haploid line of the other, attacked both *Panicum miliaceum* (a host of both species of smut) and *Panicum capillare* (a host of *Sorosporium syntherismae* only). Thus the interspecific dicaryophyte had the combined pathogenic capabilities of both species of smut.

The interspecific dicaryophytes originating from different monosporidial combinations differed strikingly in their ability to produce smut on the White Ural variety of *Panicum miliaceum* when inoculated in the field (See Table 5 and Fig. 4). The percentage of smutted heads produced by the different dicaryophytes ranged from 8.6 per cent ($J_1 \times 10C_4$) to 84.9 per cent ($J_2 \times 10D_1$). (See Fig. 4.)

Experiments in the greenhouse in which 6 varieties of *Panicum miliaceum* were inoculated with 6 interspecific combinations confirmed these differences in the pathogenicity of different interspecific dicaryophytes. The results of a series of inoculations in the greenhouse are given in table 8. The dicaryophytes arising from combinations $J_2 \times 10D_1$, $J_2 \times 10D_2$, $J_2 \times 10D_3$, and

$J_2 \times 10C_2$ produced the highest percentage of smut on all the varieties; the other two dicaryophytes produced a relatively low percentage or no smut, depending on the variety. The varieties Early Fortune and Brown Ottawa were resistant to all the dicaryophytes, the highest percentage of smut produced on these 2 varieties being between 10 and 15 per cent by the dicaryophytes that were the most pathogenic on the other varieties.

It is not surprising to find such great differences in the pathogenicity of interspecific dicaryophytes, however, because such differences have been reported in intraspecific dicaryophytes of certain other smuts (3, 19, 20).

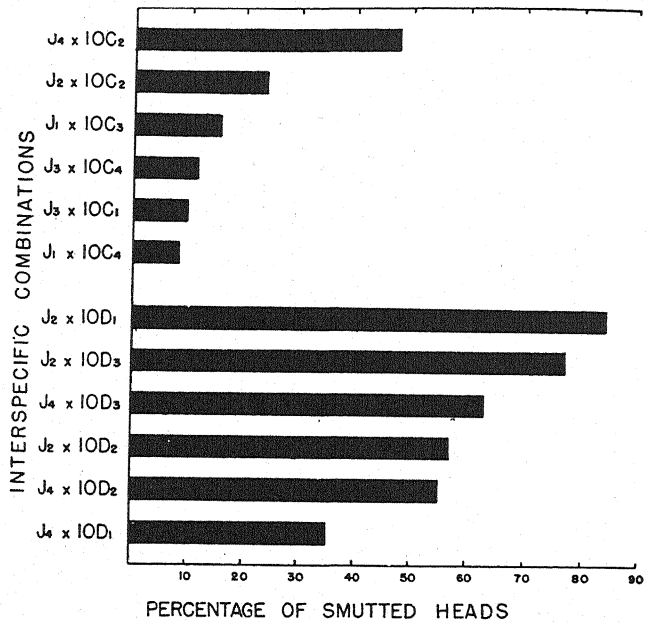



FIG. 4. Percentage smut produced on the White Ural variety of *Panicum miliaceum* by twelve interspecific dicaryophytes of *Sorosporium syntherismae* × *Sphacelotheca panici-miliacei* in the field.

The differences in the pathogenicity of dicaryophytes are important in classifying varieties as to resistance or susceptibility to smut. For example, varieties of *Panicum miliaceum* would be classed as resistant to interspecific dicaryophytes if their reaction to the 2 combinations $J_1 \times 10C_3$ and $J_3 \times 10C_1$ only was considered; but these same varieties would be classed as very susceptible on the basis of their reaction to the combination $J_2 \times 10C_2$ (Table 8).

*Other Characters.* The $F_1$ interspecific hybrid was intermediate between the two parent species in spore arrangement and also in type of sori (Fig. 3). The spores of the $F_1$ hybrid were somewhat loosely grouped but not in definite spore-balls, those of the Sorosporium parent were arranged in definite spore-balls, while those of the Sphacelotheca parent were single. The sori of the $F_1$ hybrid were ovoid to elongate and certain ones had rather definite central columellae, while others did not; the sori of the Sorosporium

parent were elongate and had no central columella; the sori of the Sphacelotheca parent were ovoid to spherical and had definite central columellae.

The cultural characters of the $f_1$ sporidial lines from interspecific cross 20 (*Sorosporium syntherismae* $J_4 \times$ *Sphacelotheca panici-miliacei* $5A_2$) were

TABLE 8.—*Percentage of smut produced by six different interspecific sporidial combinations of Sorosporium syntherismae × Sphacelotheca panici-miliacei on six varieties of Panicum miliaceum in the greenhouse*

| Variety | Interspecific combination *S. syn.* × *S. p–m.* | No. plants emerged | No. plants smutted | Percentage smutted plants |
|---|---|---|---|---|
| White Ural | $J_2 \times 10D_1$ | 46 | 32 | 69.6 |
| | $J_2 \times 10D_2$ | 52 | 24 | 46.2 |
| | $J_2 \times 10D_3$ | 40 | 23 | 57.9 |
| | $J_3 \times 10C_1$ | 45 | 1 | 2.2 |
| | $J_2 \times 10C_2$ | 42 | 35 | 83.3 |
| | $J_1 \times 10C_3$ | 34 | 6 | 12.2 |
| | Check | 54 | 0 | 0.0 |
| Early Fortune | $J_2 \times 10D_1$ | 52 | 6 | 11.5 |
| | $J_2 \times 10D_2$ | 41 | 3 | 7.3 |
| | $J_2 \times 10D_3$ | 64 | 6 | 9.3 |
| | $J_3 \times 10C_1$ | 49 | 0 | 0.0 |
| | $J_2 \times 10C_2$ | 46 | 6 | 13.0 |
| | $J_1 \times 10C_3$ | 41 | 1 | 2.4 |
| | Check | 44 | 0 | 0.0 |
| Black Voronezh | $J_2 \times 10D_1$ | 56 | 44 | 78.6 |
| | $J_2 \times 10D_2$ | 55 | 39 | 70.9 |
| | $J_2 \times 10D_3$ | 83 | 54 | 65.0 |
| | $J_3 \times 10C_1$ | 81 | 12 | 14.8 |
| | $J_2 \times 10C_2$ | 66 | 48 | 72.7 |
| | $J_1 \times 10C_3$ | 62 | 12 | 19.3 |
| | Check | 50 | 0 | 0.0 |
| Brown Ottawa | $J_2 \times 10D_1$ | 60 | 8 | 13.3 |
| | $J_2 \times 10D_2$ | 47 | 6 | 13.6 |
| | $J_2 \times 10D_3$ | 53 | 5 | 9.4 |
| | $J_3 \times 10C_1$ | 73 | 0 | 0.0 |
| | $J_2 \times 10C_2$ | 49 | 7 | 14.3 |
| | $J_1 \times 10C_3$ | 57 | 0 | 0.0 |
| | Check | 63 | 0 | 0.0 |
| Red Turghai | $J_2 \times 10D_1$ | 48 | 18 | 37.5 |
| | $J_2 \times 10D_2$ | 55 | 19 | 34.5 |
| | $J_2 \times 10D_3$ | 48 | 20 | 41.6 |
| | $J_3 \times 10C_1$ | 43 | 0 | 0.0 |
| | $J_2 \times 10C_2$ | 51 | 33 | 64.7 |
| | $J_1 \times 10C_3$ | 54 | 3 | 5.5 |
| | Check | 45 | 0 | 0.0 |
| Early Manitoba | $J_2 \times 10D_1$ | 44 | 44 | 100.0 |
| | $J_2 \times 10D_2$ | 32 | 27 | 84.4 |
| | $J_2 \times 10D_3$ | 37 | 29 | 78.8 |
| | $J_3 \times 10C_1$ | 40 | 6 | 15.0 |
| | $J_2 \times 10C_2$ | 31 | 29 | 93.5 |
| | $J_1 \times 10C_3$ | 34 | 15 | 44.1 |
| | Check | 24 | 0 | 0.0 |

compared with those of the parental lines. Certain characteristics of both parental lines were found in the $f_1$ lines, but no exact duplicate of either of the parents was found in the 18 monosporidial lines studied. This was not surprising, however, since parental types were not recovered from the intra-

specific crosses reported at the beginning of this paper. The various biotypes present in the lines studied are illustrated in figure 5. The lines

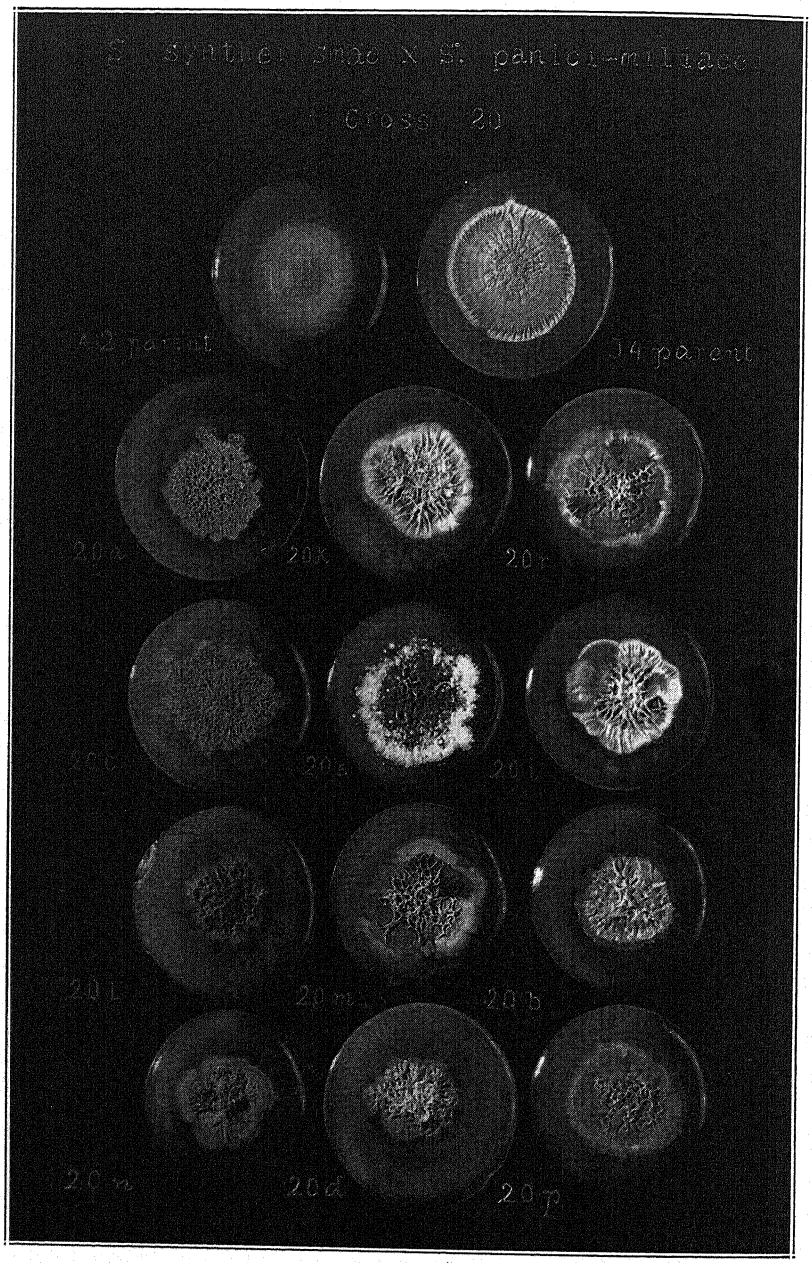



FIG. 5. Cultural races in the $f_1$ sporidial lines from an interspecific cross between *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei*.

b, i, r, and p resemble more closely the Sorosporium parent ($J_4$), and the lines a, c, and t resemble more closely the Sphacelotheca parent ($5A_2$).

### Evidence of Hybridization Occurring in Nature

Several experiments were made in which seeds of *Panicum miliaceum* were inoculated with a mixture of chlamydospores of both species of smut and with chlamydospores of each species individually. In one series of these experiments about 50 per cent smut (based on about 100 plants) was produced by each of the species and by the mixture of both species. Hybrid chlamydospores were found in two of the galls produced on plants that had been inoculated with the mixture. In both cases these hybrid spores were found in galls that contained over 50 per cent smooth spores (those of *Sphacelotheca panici-miliacei*), and there was no question that they were hybrid spores. This is further evidence that hybridization between the two species of smut could easily occur in nature where the two smuts occur in the same locality. Kammerling (12) reported that he found in nature a hybrid between *Ustilago longissima* and *U. longissima* var. *macrospora*. He, also, was able to produce experimental hybrids between these two smuts.

## SUMMARY AND CONCLUSIONS

The life cycle and nuclear condition in *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* are similar to those of most smut fungi that have been studied; the sporidia are haploid and potentially gametic, infection occurs in the seedling stage of the host, the parasitic phase is dicaryotic, mature chlamydospores are diploid, and reduction-division occurs during germination of chlamydospores.

Much the same type of variation occurs in both *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* as occurs in other smut fungi that attack graminaceous crop plants. There are numerous biotypes within each species, and new biotypes result from mutation and hybridization.

The collections of *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* studied by the writer are definitely distinct species. They differ in type of sorus as well as in size, arrangement, and markings of the chlamydospores. The writer has not collected intergrades between the two species, as reported by Zundel and others (24, 23), possibly because collections were not made over a sufficiently wide area. The results from cross inoculations with the two species demonstrated that *S. panici-miliacei* attacked only *Panicum miliaceum,* which is in agreement with reports in the literature (1, 24). But it was demonstrated that *S. syntherismae* attacks *P. miliaceum* as well as *Panicum capillare*. Neither Zundel (24) nor Barnhart (1) lists *P. miliaceum* as a host of *S. syntherismae,* although Zundel does state that certain collections on *P. miliaceum* closely resembled *S. syntherismae*. From the writer's results it appears that the abnormal specimens of Zundel (24) and others (23) might have been specimens of *S. syntherismae* or hybrids between this species and *S. panici-miliacei*. Chlamydospores of hybrids between the two species certainly fit the description of the abnormal specimens of these two investigators. Furthermore, hybridization readily occurred between the two species under experimental conditions, some of

which do not appear to be far different from conditions that might occur in nature.

The interspecific hybrid between *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* was more or less intermediate between the two parent species in many characters, including type of sorus, size of spores, markings of the spore walls, arrangement of the spores, time required for germination of the spores, and pathogenicity. Size of spores and markings of the spore walls are controlled by different factors, which appear to be inherited independently. The factor for verruculose spore walls behaves as a simple dominant over that for smooth spore walls. Fewer than 10 per cent of the $f_1$ hybrid sporidia were viable, which has been reported also for other interspecific crosses in the smut fungi (9, 10).

The interspecific dicaryophytes produced in the study were capable of attacking both *Panicum miliaceum* and *Panicum capillare*. Furthermore, there were marked differences in the pathogenicity of dicaryophytes arising from different interspecific combinations. Other investigators (3, 9, 10, 12, 19, 20, 21) have emphasized the importance of interspecific, as well as intraspecific, hybridization in giving rise to new biotypes in the smut fungi.

UNIVERSITY FARM,
ST. PAUL, MINNESOTA.

LITERATURE CITED

1. BARNHART, J. H. Revised host index of Ustilaginales. N. Amer. Flora 7: 1031–1045. 1939.
2. BAUCH, R. Über multipolare Sexualität bei *Ustilago longissima*. Arch. Protistenk. 70: 417–466. 1930.
3. CHRISTENSEN, J. J., and H. A. RODENHISER. Physiologic specialization and genetics of the smut fungi. Bot. Rev. 6: 389–425. 1940.
4. CLINTON, G. P. Ustilaginales. N. Amer. Flora 7: 1–45. 1906.
5. DAVIS, W. H. Summary of investigations with *Ustilago striaeformis* parasitizing some common grasses. Phytopath. 25: 810–817. 1935.
6. FISCHER, G. W. Two cases of haplo-lethal deficiency in *Ustilago bullata* operative against saprophytism. Mycologia 32: 275–289. 1940.
7. ———. Fundamental studies of the stripe smut of grasses (*Ustilago striaeformis*) in the Pacific Northwest. Phytopath. 30: 93–118. 1940.
8. HARPER, R. A. Nuclear phenomena in certain stages in the development of the smuts. Trans. Wis. Acad. Sci., Arts and Letters 12: 475–498. 1898.
9. HOLTON, C. S. Studies in the genetics and cytology of *Usilago avenae* and *Ustilago levis*. Minn. Agr. Exp. Stat. Tech. Bull. 87, 34 pp. 1932.
10. ———, and G. W. FISCHER. Hybridization between *Ustilago avenae* and *Ustilago perennans*. Jour. Agr. Res. [U. S.] 62: 121–128. 1941.
11. JOHNSON, H. W., H. A. RODENHISER, and C. L. LEFEBVRE. Two types of fall Panicum smut. Jour. Agr. Res. [U. S.] 61: 865–875. 1940.
12. KAMMERLING, H. Ueber Geschlechterverteilung und Bastardierung von *Ustilago longissima* und ihrer Varietät *macrospora*. Zeitschr. Bot. 22: 113–142. 1929.
13. KERNKAMP, M. F. Genetic and environmental factors affecting growth types of *Ustilago zeae*. Phytopath. 29: 473–484. 1939.
14. ———, and M. A. PETTY. Variation in the germination of chlamydospores of *Ustilago zeae*. Phytopath. 31: 333–340. 1941.
15. MARTIN, W. J., and M. F. KERNKAMP. Variation in cultures of certain grass smuts. Phytopath. 31: 761–763. 1941.
16. NORTON, J. B. S. A study of the Kansas Ustilagineae, especially with reference to their germination. Trans. Acad. Sci. (St. Louis) 7: 229–241. 1896.
17. RODENHISER, H. A. Studies on the possible origin of physiologic forms of *Sphacelotheca sorghi* and *S. cruenta*. Jour. Agr. Res. [U. S.] 49: 1069–1086. 1934.

18. SAMPSON, KATHLEEN. Presidential address. Life cycles of smut fungi. Trans. Brit. Mycol. Soc. 23: 1–23. 1939.
19. STAKMAN, E. C. The problem of specialization and variation in phytopathogenic fungi. Genetica 18: 372–389. 1936.
20. ————, J. J. CHRISTENSEN, C. J. EIDE, and B. PETURSON. Mutation and hybridization in *Ustilago zeae*. Minn. Agr. Exp. Stat. Tech. Bull. 65, 108 pp. 1929.
21. VAHEEDUDDIN, S. Hybridization between *Sphacelotheca sorghi* and *Sorosporium reilianum*. (Abstract) Phytopath. 26: 111. 1936.
22. WANG, D. T. Contributions à l'étude des Ustilaginées. (Cytologie du parasite et pathologie de la cellule hôte.) Le Botaniste 26: 539–670. 1934.
23. WINTER, G. Pilze. *In* Rabenhorst's Kryptogamen-Flora. Band I Abt. I. Schizomyceten, Saccharomyceten, und Basidiomyceten. (Leipzig.) 1884.
24. ZUNDEL, G. L. I. Additions and corrections to the Ustilaginales. N. Amer. Flora 7: 971–1030. 1939.

# SEVERITY OF CURLY TOP IN TOBACCO AFFECTED BY SITE OF INOCULATION

W. C. Price

(Accepted for publication November 6, 1942)

Evidence for passive immunization of tobacco (*Nicotiana tabacum* L.) from the virus of sugar-beet curly top (*Chlorogenus eutetticola* H.) was reported by Wallace (20) in 1940. It was observed that grafting with cions from tobacco plants that had recovered from curly top transmitted only a mild disease, whereas the insect vector (*Eutettix tenellus* (Baker)) transmitted a severe disease from the same source plants. Wallace concluded that the grafting process transmitted protective substances along with the virus, whereas the insect vector transmitted only the virus. The studies herewith presented dealt with the effect of route of infection on severity of curly-top symptoms in tobacco. In these it was found that the grafting process does not always transmit a mild disease from recovered plants and that the insect vector may transmit either a mild or a severe disease, depending upon the portion of the test plant on which it is allowed to feed. The results, therefore, do not support the antibody hypothesis, but rather indicate that the explanation for production of only mild symptoms by grafting must be sought elsewhere.

## REVIEW OF LITERATURE

Of the many species of plants susceptible to curly-top virus, tomato (*Lycopersicon esculentum* Mill. and *L. pimpinellifolium* Mill.) and tobacco (*Nicotiana tabacum*) have been shown to recover from the acute form of disease produced by this virus. When curly-top virus is taken to tomato, it induces clearing of veins of the newly formed leaflets and severe curling and distortion of the youngest leaves; affected plants gradually wilt, turn yellow, and die within a few weeks after infection (4). It was observed by Lesley (7), however, that tomato plants in an advanced stage of curly top sometimes recovered, especially in late summer and fall. This recovery was probably due to the escape of portions of the tomato plants from the curly-top virus, since some of the new shoots from recovered plants later became severely diseased and since, in one instance, exposure of a recovered plant to viruliferous insects resulted in development of severe curly top in this plant. A different type of recovery was observed by Lesley and Wallace (8) in plants of certain races of tomato. In some of these plants, relatively healthy shoots arose from the leaf axils and the plants then grew almost normally until maturity, although they showed mild symptoms of disease and continued to harbor the curly-top virus. In most instances, reinoculation of the recovered plants failed to induce further symptoms. In a few cases, however, recovered plants relapsed and suffered a second attack of curly top.

Sugar-beet curly-top virus was first transmitted to tobacco by Severin (13) in 1929. In 1936 Bennett and Esau (3) reported that tobacco plants infected with the virus showed a marked recovery from the initial disease, the new growth appearing healthy or developing only mild symptoms. The recovery was apparently due to a reaction on the part of the plant itself and not to an attenuation of virus. Wallace (19) confirmed the observations of Bennett and Esau and stated that the new, healthy-appearing growth may arise either from the axillary or terminal buds. He showed further that recovered tobacco plants may be grown from cuttings without again showing severe symptoms, even though reinoculated with the virus. This acquired immunity from curly top is thus similar to that observed in tobacco ring spot and certain other plant virus diseases (10).

Wallace (20) not only found, as previously mentioned, a difference between symptoms following insect transmission and those resulting from grafting with cions from recovered plants, but he also observed that the severity of disease induced by grafting was dependent upon the stage of infection of plants from which cions were taken. Severe symptoms developed in healthy tobacco plants grafted with scions taken from plants 5 days after they had been inoculated with curly-top virus, mild symptoms were obtained on plants grafted with cions taken from plants exposed 20 days previously to viruliferous insects, and symptoms of intermediate severity developed when cions from plants inoculated 10 and 15 days previously were used. These results led to the conclusion that "the reaction leading to recovery took place at an early stage following infection and that it was completed or well advanced before the plants began to recover." The results were thus taken as corroborative evidence that protective substances were transmitted from recovered plants by means of grafting.

Still further evidence for the antibody hypothesis was reported by Wallace (21) in 1942. According to him, the clonal progeny of tomato plants that were grafted with cions from recovered tobacco plants showed a high degree of protection from curly top when grown under field conditions. Such grafted plants, when reinoculated with curly-top virus, were either unaffected, mildly affected, or severely affected, depending upon the strain of virus used for reinoculation. Thus, while grafting with cions from recovered tobacco plants gave protection against the strain of virus originally used to inoculate the tobacco plants from which cions were taken, it did not give protection against other closely related strains of the virus.

## MATERIALS AND METHODS

In the first attempts to transmit sugar-beet curly-top virus to Turkish tobacco, the virus either was not transmitted or failed to induce obvious signs of disease. The strain of virus available at that time was, therefore, not suitable for the purpose. Later, a strain that did induce severe symptoms in Turkish tobacco was kindly supplied by Dr. Wallace. This strain apparently is identical with the one with which he worked and has been used in all the experiments to be reported.

Turkish tobacco and tomato plants were, for the most part, grown in 6-inch porous clay pots in a greenhouse held above 70° F. They grew more rapidly in the late spring, summer, and early fall than during the winter months, but the symptoms induced on them did not appear to be correlated with the season. The greenhouse was fumigated at frequent intervals to control insects.

The leaf hoppers used for transmission of the sugar-beet curly-top virus were maintained on healthy or curly-top-diseased sugar beets in a separate greenhouse or in a laboratory window until placed on tobacco or tomato test plants. They were handled by means of a suction tube similar to that devised by Kunkel (6) and caged on the test plants or portions of them by means of celluloid cages similar to those described by Shapovalov (14). *Eutettix tenellus* will feed and survive on tobacco only for about a day. It was, therefore, the practice in this work to place from 6 to 15 leaf hoppers on the test tobacco and tomato plants for one day and then remove them to sugar beets for at least a day before again using them for tobacco or tomato. In this way an infective colony could be used for a number of successive transmissions.

Two types of grafts were employed. One was the cleft graft and the other will be referred to as a lateral graft. In the former type the cion consisted of a portion of the stem from 3 to 5 inches in length, with most of the leaves removed, and cut to a wedge shape on the basal end. For the stock vigorous plants were decapitated 6 or more inches above the soil level and the stub split with a sharp knife for a distance of about 2 inches. The cion was inserted into the split stem and the two bound together. A paper bag was then placed over the top of the plant and left on for 1 or 2 days. In the lateral graft the cion was a 1-inch slice of stem, containing a bud, from which the pith had been removed or, in some cases, a section of stem, with leaves, from the top of a plant, sliced diagonally at the base. The cion was bound to a section of stem of the stock from which the epidermis had been removed or scarified. The binding material, "Sterilastic," was removed or cut away after 2 or 3 weeks or was left on and allowed to disintegrate.

## DESCRIPTION OF SYMPTOMS

The terms severe, mild, moderate, and moderately severe will be used to describe the symptoms obtained in tobacco in the experiments to be reported. These are relative terms used merely to indicate the degree of severity of symptoms. They refer to syndromes, which may be described as follows:

*Severe:* The first sign of disease is clearing of veins in young, partly expanded, apical leaves. All the main veins become bright yellow and thus make the venation conspicuous. Leaves showing this symptom usually curl upward at the basal edges. Leaves produced later turn downward from the edges. Usually 8 to 10 or more leaves show this downward curling, most of them without marked clearing of veins. The veins expand less than the

interveinal tissue, giving the leaf a decidedly puckered appearance. Many of the leaves are about 1/20 their normal size and rolled into compact masses or balls. They frequently retain their normal green color; sometimes they are even darker green than normal. Older leaves, those first affected, or those that were more fully expanded at the time they were invaded, may yellow and die. Internodes are markedly shortened; a section of stem 1 inch long may bear 5 or 6 leaves as compared with only 1 or less for the normal plant. Thus, the apex of the plant becomes rosetted. The stem may grow more on one side than on the opposite, curling downward or becoming twisted and distorted. As the plant matures, the rosetted appearance may become less marked due to some unfolding of affected leaves and lengthening of the shortened internodes, but the affected portions never appear normal or even nearly so. Axillary buds sometimes are stimulated to grow, but shoots produced from them remain small, become markedly curled and distorted, and often die. When the plant is infected late, the flower stalk may show similar symptoms. The flowers are bunched, the sepals crinkled or puckered, and the corolla misshaped. After a variable period of from 1 to 3 months, an axillary bud or the terminal bud may send out a healthy-appearing shoot. This shoot continues to grow and never shows the severe symptoms characteristic of the onset stage, although it may at times produce leaves with mild clearing of veins, curling, or puckering. The plant has thus recovered from the acute form of the disease.

*Mild:* The symptoms first appear as clearing of veins, as in the severe type, but the clearing usually is not so pronounced. The leaf edges curl upward at the base. Leaves so affected may later curl downward and show crinkling of the tissues between the lateral veins. They retain their normal color or may become slightly darker green than normal. Usually only 2 or 3 leaves show such manifestations; they are reduced only slightly in size, perhaps to $\frac{3}{4}$ normal, and become more nearly normal as they mature. The internodes are not appreciably shortened and the plant does not assume a rosetted appearance. After the initial shock the new growth appears almost normal. It may often be differentiated from healthy growth by its slightly darker color and by the appearance of crinkled or puckered areas between lateral veins.

*Moderate and Moderately Severe:* There are various gradations between the two extremes, mild and severe, of symptom expression. The number of leaves affected may vary from 2 to 15 or more. Affected leaves may be curled more than in the mild type but less than in the severe. Also, the degree of shortening of the internodes may vary. The terms moderate and moderately severe are used to indicate degrees of severity of symptoms between the mild and severe types. Whether the disease picture be classified as severe or moderately severe, as mild or moderate, is a matter of judgment. An affected plant may be classified as moderate on one occasion and mild or moderately severe, or even severe, on the next examination; that is to say, the plants may have become more or less severely diseased in the

interval between examinations. For this reason, affected plants should be examined at frequent intervals over a period of 2 or more weeks after they first show symptoms before being classified according to symptom type.

## EXPERIMENTAL

### Tests with Turkish Tobacco

*Symptoms Produced by Cleft-grafting with Cions from Newly Infected and from Recovered Plants.* Turkish tobacco plants, grown in 6-inch pots to a height of 18 to 24 inches in one test and 12 to 16 inches in another, were cut back to a height of about 8 inches and cleft-grafted either with cions from plants that had recovered from curly top or with cions from recently diseased plants (exposed 10 days previously to viruliferous insects). In the first test symptoms appeared on some plants 8 days after grafting but failed to appear on more than half of the plants within one month after

TABLE 1.—*Symptoms produced in Turkish tobacco plants after cleft-grafting with cions from recently diseased and recovered plants*

| | | Plant No. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Test 1 | | | | | | | | | | | | | |
| R[a] | B | +[b] | +++ | – | – | – | – | +++ | – | – | +++ | +++ | – |
| | A | + | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| D | B | ++++ | – | – | +++ | – | ++++ | +++ | – | + | – | – | – |
| | A | + | ++++ | ++++ | ++++ | ++++ | + | +++ | + | ++++ | ++++ | ++++ | ++++ |
| Test 2 | | | | | | | | | | | | | |
| R ..... | | +++ | ++ | + | + | – | ++ | +++ | + | – | | | |
| D ..... | | ++++ | ++++ | + | +++ | +++ | – | ++++ | – | – | | | |

[a] R, cions from recovered plants; D, cions from recently diseased plants; B, before being pruned back; A, after being pruned back.

[b] ++++, severe symptoms; +++, moderately severe symptoms; ++, moderate symptoms; +, mild symptoms; –, no symptoms.

grafting. The plants were therefore pruned back to the first bud or shoot above ground level and held under observation for another month. Plants in the second test were grown for a period of 6 weeks after grafting and observed for signs of disease at frequent intervals during this period. The symptoms produced in the two tests are summarized in table 1. The results were unexpected, on the basis of what had previously been reported (20). There was no clear-cut difference between symptoms induced by the two types of cions, although there seemed to be a tendency for cions from recently diseased plants to induce somewhat more severe symptoms on the average than those from recovered plants. The failure of many plants to show symptoms at all was obviously due to the failure of virus to reach the growing tips where its effects could become apparent. If antibodies or protective substances were transferred from cion to stock, it would seem that the stock should be protected and show only mild symptoms. Since this was not the case, the results could not be construed as bringing evidence for the presence of such substances.

Plants that were cleft-grafted with scions from recovered or recently diseased plants usually developed more than one axillary shoot. In many instances different shoots on the same grafted plant showed different symptoms. While some shoots developed severe symptoms, others on the same plant developed none at all or only mild symptoms.

On many occasions, tobacco plants that had fully recovered from a severe attack of curly top were pruned back and the axillary buds forced into growth. This growth in some cases appeared normal, but in most instances it showed mild symptoms of curly top for a short time and then appeared practically normal. In no instance did severe curly-top symptoms develop when fully recovered tobacco plants were pruned back.

The behavior of one plant, number 7 of test 2, grafted with a cion from a recently diseased plant, is worthy of further comment. The cion made

TABLE 2.—*Symptoms produced in Turkish tobacco plants after grafting laterally with cions from recovered and recently infected plants*[a]

| Test No. | | Plant No. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | R | ++++ | ++ | ++ | ++ | − | − | +++ | ++ | +++ | ++ | +++ | +++ |
| | D | ++++ | +++ | +++ | ++++ | ++++ | +++ | ++++ | ++ | − | ++++ | ++++ | +++ |
| 2 | R | + | − | + | + | − | ++++ | + | − | − | + | + | − |
| | D | ++++ | ++ | + | ++++ | ++ | + | + | − | + | − | − | + |
| 3 | $D_1$ | +++ | − | − | + | − | ++ | ++++ | − | + | ++++ | ++ | + |
| | $D_2$ | − | ++++ | − | − | − | | + | +++ | − | − | − | − |
| | $D_3$ | − | − | ++++ | +++ | ++++ | − | − | − | +++ | − | − | − |

[a] For explanation of symbols, see footnotes to table 1. $D_1$, $D_2$, and $D_3$, cions from plants 20, 15, and 10 days, respectively, after exposure to viruliferous insects.

a quick union with the stock and grew rapidly. Its growth apparently inhibited the production of axillary shoots, since these developed very slowly. The symptoms produced on the cion were at first severe, then became mild, and finally disappeared as the cion recovered. Six weeks after grafting the cion had grown to a height of 11 inches, most of it being composed of healthy-appearing foliage. Two shoots had grown from axillary buds at the base and both of these showed severe symptoms. The potential supply of antibodies in this recovered cion was indeed large as compared with the supply in a newly grafted cion. If such substances were present and capable of moving from one part of a plant to another, it is surprising that they did not inhibit the symptoms on the axillary shoots produced after the cion had recovered. It is to be inferred that such substances either were not present or, if present, were incapable of moving in this instance.

*Symptoms Produced by Lateral Grafting with Cions from Newly Infected and from Recovered Plants.* The possibility existed that the rapid growth of axillary shoots following cleft-grafting in some way may have overcome the influence of any protective substances that had been transmitted through the graft union. The lateral graft, which does not stimulate

axillary buds to grow, was used in a series of tests with the results summarized in table 2. Here again the data indicated a slight difference between symptoms produced by grafting with cions from recovered plants and from recently infected plants. The difference, however, was not consistent and can possibly be attributed largely to chance. Moreover, it is apparent that many plants grafted with cions from recovered plants came down with severe or moderately severe symptoms, while a smaller number showed mild symptoms. It could not be concluded that the experiment demonstrated the movement of antibodies from cion to stock.

*Attempt to Isolate Mild and Severe Strains by Selection.* In contrast to the variable symptoms induced by grafting, plants inoculated by allowing viruliferous insects to feed on them invariably came down with severe curly top. Was this difference due to the transmission of antibodies by the grafting process or was it due, for example, to the presence of a mixture of virus strains in the inoculum? It could be reasoned that, following grafting, one or another strain might predominate fortuitously and that, on the other hand, the vector tended to select the more severe strain without entirely eliminating the others. To test this possibility, an experiment was designed to see whether or not severe and mild strains could be obtained by a process of selection and elimination over a period of time.

Two plants from a previous experiment were selected as a source of cions; one had developed mild symptoms after grafting and the other had developed severe symptoms. Six cions were taken from each plant and grafted separately to Turkish tobacco plants. The cions from the severely affected plant produced severe symptoms in one stock and mild or moderate ones in the others; the plant with severe symptoms was selected for further grafting. The cions from the mildly affected plant produced mild symptoms in one plant, moderate symptoms in 3, and none in 2; the mildly affected stock was selected for additional grafting. This process was continued until each selection had been passed through 5 successive transfers by grafting. At the end of this period there was no evidence that there had been a selection of strains from a complex, and the 2 isolates showed about the same variation in symptoms as they had in the beginning. This experiment, therefore, brought no evidence that the difference between symptoms produced following grafting and those following insect transmission could be accounted for on the basis of a mixture of virus strains.

*Influence of Source Plants and Position from Which Cions Were Taken.* While the data so far presented indicated a significant difference between transmission by grafting and by means of the insect, this difference was by no means so clear-cut as that obtained by Wallace (20). Moreover, the results with grafting were erratic; grafting with cions from recently diseased or recovered plants might result either in a mild or a severe disease, and it was impossible to predict ahead of time which result would be obtained. If proof of the antibody hypothesis were to be secured, it was necessary to obtain consistent results. What factors could account for the

variation? Was it possible that different recovered plants or parts of these plants differed in their antibody content or in their ability to transmit antibodies to a plant on which they were grafted? An experiment designed to give an answer to the latter question was carried out on two different occasions. Recovered plants were selected and 5 cions were taken from each, 1 from the top of the plant, 1 from near the base, and 3 from intermediate positions. Each cion was cleft-grafted to a large healthy plant in a 6-inch pot. Table 3 summarizes the results obtained. The 5 cions from one recovered plant all transmitted a mild disease; the 5 cions from 2 other recovered plants transmitted a severe disease in each instance; the 5 cions from the other 13 plants transmitted severe, mild, or intermediate symptoms indiscriminately. There appeared to be no correlation between cion

TABLE 3.—*Symptoms produced in Turkish tobacco plants after cleft-grafting with cions from different portions of recovered plants*[a]

| Cion position | | Plant No. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Top | A | ++ | +++ | ++++ | ++ | + | ++++ | | | | |
| | B | + | ++ | + | + | + | ++++ | | | | |
| | C | − | + | +++ | +++ | +++ | + | | | | |
| | D | ++++ | +++ | + | +++ | ++ | ++ | | | | |
| Base | E | ++ | +++ | +++ | ++ | ++++ | − | | | | |
| Top | A | + | + | ++++ | ++++ | + | ++ | ++++ | ++++ | +++ | + |
| | B | + | + | ++ | + | + | + | ++++ | ++++ | ++++ | ++++ |
| | C | + | + | ++++ | ++ | ++ | + | ++++ | ++++ | + | ++++ |
| | D | ++++ | + | ++++ | ++ | ++ | + | ++++ | ++++ | ++++ | ++++ |
| Base | E | ++++ | + | ++++ | ++++ | + | ++++ | ++++ | ++++ | + | ++++ |

[a] For explanation of symbols, see footnotes to table 1.

position and type of disease transmitted. It was concluded that some as yet unknown factor was responsible for the variation observed.

*Rate of Appearance of Symptoms after Grafting.* It has been shown by Bennett (1) and confirmed in the present work that from 6 to 15 days are required for curly-top virus to pass a graft union in Turkish tobacco. Under normal conditions the plant grows considerably in this period. Thus, the question arose as to whether the variation in symptoms observed might not be due to age or size of plant at the time of invasion by the virus. The daily records of time of appearance and severity of symptoms from 2 experiments in which healthy Turkish tobacco plants were grafted with cions from recovered plants furnished a partial answer to this question. These records are summarized in table 4. There was but little correlation between severity of symptoms and time when the symptoms first appeared.

It is of interest that when days after grafting were plotted against the number of plants showing symptoms the data followed an S-shaped curve. Part of the variation in time of appearance of symptoms was undoubtedly due to the variation in time required for virus to pass the graft union; part was probably due to the variation in rate of upward movement and distance traveled once the union was passed.

TABLE 4.—*Time required for symptoms to appear in tobacco plants after grafting with cions from recovered plants*[a]

| Days after grafting when symptoms first appeared | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 19 | 20 | 24 | 30 or more |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Symptom type | +++<br>+++<br>+++ | ++++<br>+<br>++ | ++ | ++++<br>+++ | +<br>+<br>+<br>++<br>++<br>++++ | +++<br>++++<br>+<br>+ | ++<br>++++<br>+<br>++++ | +<br>+<br>++<br>+<br>+<br>+++<br>+ | +<br>++<br>++<br>+<br>+++<br>++++<br>+<br>+ | +++ | +<br>+<br>+ | +<br>+++<br>+<br>+<br>+<br>+++<br>++ |

[a] For explanation of symbols, see footnote to table 1.

*Insect Transmission to Large Plants.* Additional evidence that variation in symptoms was not due to age or size of plant at time of infection was obtained by exposing large Turkish tobacco plants to viruliferous insects. Eighteen large plants, 6 of which were trimmed as though for cleft-grafting, were exposed. Seven of the untopped plants and 3 of the topped ones came down with severe symptoms, while 5 of the former and 3 of the latter failed to become infected at all.

*Cleft-grafting Old Plants.* Still further evidence that age of plant has little influence on severity of symptoms was obtained by cleft-grafting 12 old Turkish tobacco plants at a point between $2\frac{1}{2}$ and 3 feet above the soil level. As usual, the symptoms varied with the individual plants. They were severe on 6, moderately severe on 3, moderate on 1, and mild on 2 plants.

*Effect of Forcing Grafted Plants into Rapid Growth.* It was thought possible that there might be a difference between symptoms produced by rapid-growing and slow-growing plants after grafting with cions from

TABLE 5.—*Symptoms produced in plants cleft-grafted with cions from recovered plants and subsequently forced into rapid growth or not forced*[a]

| | Plant No. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Test 1 | | | | | | | | | | | | |
| Forced ... | + | ++++ | ++++ | + | – | ++++ | ++++ | ++++ | ++++ | +++ | ++ | + |
| Not forced | + | +++ | ++++ | – | ++++ | + | ++++ | ++++ | ++++ | ++++ | ++++ | – |
| Test 2 | | | | | | | | | | | | |
| Forced ... | ++ | + | ++++ | ++ | ++++ | ++++ | + | +++ | ++ | + | + | – |
| Not forced | – | – | + | + | +++ | – | + | ++++ | – | + | + | – |

[a] For explanation of symbols, see footnote to table 1.

recovered plants. To test this, stock plants were grown in 6-inch pots to a height of about 3 feet; half were then repotted in 8-inch pots, while the remainder were left in 6-inch pots. All of the plants were cut back to a point about 12 inches above the soil level, then cleft-grafted with cions from recovered tobacco plants, and held for observation for 2 months. The symptoms produced are recorded in table 5. There was no consistent difference between the symptoms produced on the two groups of test plants. It could not be concluded that variation in symptoms of individual plants was correlated with variation in growth rate.

*Symptoms Produced when Viruliferous Insects Were Fed on Various Portions of Turkish Tobacco Plants.* As mentioned previously, when

TABLE 6.—*Symptoms produced on plants exposed to viruliferous insects and on plants inoculated by grafting*[a]

| Test | | Plant No. | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | Insects on tip leaves | – | ++++ | ++++ | ++++ | ++++ | – |
| | Insects on single leaf | ++++ | – | + | ++++ | – | ++++ |
| | Insects on stem | – | – | – | – | – | – |
| | Grafted on stem | ++ | + | + | – | + | – |
| 2 | Insects on tip leaves | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| | Insects on single leaf | – | – | – | – | +++ | + |
| | Insects on stem | – | – | + | – | + | – |
| | Grafted | + | – | – | + | + | ++ |
| 3 | Insects on tip leaves | – | – | ++++ | – | ++++ | ++++ |
| | Insects on single leaf | +++ | – | ++++ | – | – | – |
| | Insects on stem | – | – | ++ | – | ++ | – |
| | Grafted | – | – | – | – | – | – |

[a] For explanation of symbols, see footnotes to table 1.

tobacco plants were grafted with cions from diseased or recovered plants, 6 days or more were required for virus to pass from the cion into the stock. During this period the grafted plants continued to grow and the terminal bud got further and further away from the graft union. Thus, in order to cause symptoms, it was necessary for the virus to move upward several inches at least. On the other hand, when transmission was by means of the insect vector, the virus might be introduced into tissues very near the growing point and thus might infect the growing point quickly. It has been shown by Bennett (2) that, while downward movement of the curly-top virus is rapid in tobacco plants, upward movement is slow. To compare the effects of insect transmission and graft transmission, it was necessary to keep these facts in mind. To draw conclusions from such a comparison, it was essential that the conditions under which the two types of transmissions were employed should be as nearly alike as possible. This could be done by delaying the insect feeding for at least the minimum period required for virus to cross the graft union and by confining the insects to a portion of the plant at the same relative distance from the terminal bud as the graft.

A number of experiments were conducted in which this was done. In each experiment the plants were divided into groups. Viruliferous insects were allowed to feed in the usual manner on one group; they were confined to a single leaf 3 or 4 inches below the tip in a second group; they were confined to a 2-inch portion of the stem 3 or 4 inches below the terminal bud in a third group. Another lot of plants was grafted laterally with cions from plants that had recovered from curly top. The symptoms induced in the experiments are summarized in table 6. Plants exposed to viruliferous

FIG. 1. Turkish tobacco plants infected with curly-top virus. The plant on the left, showing moderate symptoms, was infected by allowing viruliferous insects to feed on a 2-inch section of stem 6 inches above the soil level and about 3 inches below the terminal bud 39 days before the picture was taken. The plant on the right, showing severe symptoms, was infected by allowing viruliferous insects to feed on its apical leaves 39 days before the photograph was taken. (Photograph by J. A. Carlile.)

insects in the usual manner either developed severe symptoms or failed to become infected at all. Those inoculated by grafting showed the usual variation in symptom expression. Three of the plants inoculated by insects feeding on a single leaf or a 2-inch portion of the stem showed mild symptoms only, 2 showed moderate, 2 moderately severe, and 4 severe symptoms, while 24 either failed to become infected or failed to allow virus to move to the tip before the plants had matured. The results obtained by confining

the insects to portions of the plant well below the terminal bud were, therefore, comparable to those obtained with the grafting process (Fig. 1). The conclusion to be drawn was obvious. If the insects were capable of transmitting either a mild or a severe disease, depending upon the portion of the plant upon which they fed, then it could hardly be reasoned that the insects transmitted only the virus, while the grafting process transmitted both virus and protective substances. That such substances may be produced in tobacco plants infected with curly-top virus is certainly a possibility, but the evidence available is not sufficient to justify the conclusion that they exist.

### Experiments with Tobacco Cions on Tomato Stocks

Failure to demonstrate antibodies in tobacco plants grafted with tobacco cions led to a test of Wallace's (21) conclusion that tomato plants could be passively immunized from curly top by grafting them with cions from tobacco plants that had recovered from the disease. Of 44 tomato plants grafted either terminally or laterally with cions from recovered tobacco plants, 29 became severely diseased. In many of these the symptoms were much delayed; a few plants first showed symptoms when they had nearly reached maturity. In some, however, the plants were attacked early and promptly died. The remaining plants, 15 in number, failed to show symptoms before they matured. The results with the grafted plants were comparable to those obtained in plants infected by means of the insect vector and in those grafted with cions from diseased tomatoes. Of 27 plants exposed to viruliferous insects, 10 developed a severe disease and 17 apparently escaped infection. Of 34 plants grafted with cions from diseased tomatoes, 12 became severely diseased and 22 showed no symptoms whatever. All the tomato cions died but apparently not before some of them made a union with the stock and transmitted the virus.

It is of interest in this connection that Shapovalov (15) likewise found difficulty in getting diseased tomato cions to form a union with healthy tomato stock, and he was able to transmit the virus to only one of 13 plants approach-grafted to severely diseased plants. However, he did obtain transmission across the graft union in 67 per cent of the cases when the graft was made prior to or simultaneously with exposure of the cion to viruliferous insects.

The reason for the discrepancy between the writer's results with tomato and those of Wallace is not clear. It is possible that growing conditions or other environmental influences may have an effect on immunization of tomato plants by grafting with cions from recovered tobacco plants. It is obvious from the above results that, of the 44 grafted plants, 29 not only were not immunized but actually succumbed to the virus introduced by grafting.

The results of these experiments with tomato stocks, like those of experiments involving tobacco stocks, failed to bring evidence tending to justify an assumption that antibodies were produced by infected plants.

## DISCUSSION

The conclusion of Wallace (20) that tobacco plants acquire an immunity from the curly-top disease is supported by results obtained in this study: Turkish tobacco plants suffering from an acute attack of curly top invariably recovered from the disease but did not lose the virus. They did not again develop severe symptoms when pruned back or reproduced by cuttings and forced into rapid growth. Reinoculation of recovered plants either by means of insects or by grafting did not induce further symptoms in them.

On the other hand, the work here reported does not support Wallace's hypothesis that protective substances are transmitted from recovered to healthy plants by grafting. Indeed, no direct evidence was obtained that such substances are produced at all by affected plants. The fact that either a mild or a severe type of disease resulted from insect transmission, depending upon the portion of the plant on which the insect fed, clearly indicates that the mild symptoms induced in some plants by grafting with cions of recovered plants was due to the point of inoculation, not necessarily nor probably to antibodies that might have been carried by the cion.

What then is the true explanation for the production of mild symptoms in some plants and severe symptoms in others after grafting with cions from recovered plants? It is possible to derive an hypothesis that fits the known facts much better than the antibody hypothesis. When the insect vector feeds on tissues near the growing point, it injects a relatively large quantity of virus into or close to those parts of the plant that are subject to malformation; that is, into immature or embryonic tissue. The virus has only a short distance to travel and it arrives in quantity before the tissues have an opportunity to set up a defense against it. On the other hand, when the virus is introduced into a plant some distance below the growing point, either by means of the vector or by grafting, it moves upward slowly and apparently with difficulty (2). Moreover, this movement is erratic, being more rapid in some plants than in others. If only minute quantities of virus reach the immature tissues in the terminal or lateral buds, these tissues are able to set up a defense against it and thus be partly or completely protected against the effects of additional quantities of virus to which they may be subjected. The completeness of the defense might depend upon the original dose of virus and the rapidity with which this dose is increased. If the original dose is large and quickly followed by increase, the tissues may be able only to overcome partly its effects. But, if the original amount to which the embryonic tissues are subjected is small and increase is slow, then they may suffer only slightly from its effects.

The variation in symptoms produced by grafting with cions from recovered plants would thus be explained on the basis of variation in the quantity of virus that moved from the union to growing points of the plant. This variation in the quantity of virus that moved would explain why one axillary shoot on a terminally grafted plant became severely diseased and another on the same plant showed only mild or moderate symptoms.

If it be assumed that tobacco plants recovered from curly top contain less virus than those that are severely diseased, as in the case of tobacco-ring-spot disease (9), then it is possible to explain the tendency for grafting with cions from recently diseased plants to induce, on the average, more severe symptoms than cions from recovered plants. The lower virus content in the latter case would increase the probability that the growing point of the grafted plant would receive a smaller quantity of virus. Hence, there would be a greater probability that such plants would show less severe symptoms than those grafted with cions from recently diseased plants.

There are few, if any, facts available on which to base a conjecture of the nature of the defense mechanism. By analogy with what is known about the nature of acquired immunity from virus disease in animals, the most obvious suggestion is that antibodies are involved. However, the mechanism may be of an entirely different nature, and the production of severe symptoms in plants exposed, through grafting, to any antibodies that might pass a graft union tends, if anything, to indicate that it is.

The correlation between the route of inoculation and the severity of symptoms produced in curly top of tobacco is analogous to the correlation between portal of entry of virus and severity of disease in many animal virus diseases. A few such cases will suffice for illustrative purposes. Theiler (17) was able to infect mice with yellow-fever virus by inoculation directly into the brain. Virus isolated from the brains of infected mice was extremely virulent for mice when inoculated intracerebrally and usually resulted in death. The same virus inoculated subcutaneously, intradermally, intramuscularly, or intraperitoneally seldom resulted in illness or death but often produced an immunity from a subsequent intracerebral inoculation. The mouse-passage strain of virus was low in virulence for monkeys. Sellards (12) reported that, when this strain of virus was introduced intraperitoneally into monkeys, it caused no ill effects but immunized the monkeys against a typical strain of yellow-fever virus. On the contrary, inoculation of the same virus into the brain of monkeys resulted in paralysis and death within a few days. It was shown by TenBroeck and Merrill (16) that rabbits usually were killed by intracerebral inoculation with the eastern strain of equine encephalomyelitis virus, but that rabbits given intracutaneous or subcutaneous injections of the same virus usually recovered and became immune from intracerebral inoculation. According to Rivers and Schwentker (11), large amounts of psittacosis virus could be introduced intravenously and intramuscularly into monkeys without causing serious ill effects, but only small amounts of the same virus introduced intratracheally were required to produce psittacosis pneumonia. It was reported by Traub (18) that intracerebral inoculation of mice with the virus of lymphocytic choriomeningitis usually resulted in convulsions followed by death, but that inoculation with the same virus by the intranasal, intraperitoneal, or intracutaneous route not only failed to cause death but resulted in an immunity often associated with persistence of virus. King

(5) has stated that the effect on mice of a fixed strain of equine encephalomyelitis virus—that is, one passed serially through the brains of pigeons—was dependent upon the route of inoculation. He listed these routes according to their effectiveness in the following descending order: intracerebral, intraocular or intranasal, intravenous, intraperitoneal, intramuscular, subcutaneous.

Because of the difference in the structure and physiology of plants and animals, this apparent similarity in relative effectiveness of different modes of inoculation between these animal virus diseases and curly top in tobacco may be superficial and of no particular significance. It is, however, suggestive of some fundamental characteristic or mechanism common to all these virus diseases, particularly when it is remembered that curly-top virus is confined almost entirely, if not entirely, to the phloem of diseased plants (1) and that all the animal viruses mentioned are specialized for some particular tissue or tissues within the animal body.

## SUMMARY

Grafting healthy Turkish tobacco plants with cions from tobacco plants recently infected with sugar-beet curly-top virus resulted sometimes in severe symptoms, sometimes in mild symptoms, and sometimes in symptoms of intermediate severity. Similar results were obtained when tobacco plants were grafted with cions from tobacco plants that had recovered from curly top. On the average, cions from recently diseased plants caused somewhat more severe symptoms than cions from recovered plants, but the difference was not consistent.

Grafting healthy tomato plants with cions from recovered tobacco plants resulted in production of severe symptoms, usually ending in death, or in complete absence of symptoms. In the latter case the virus either failed to pass the graft union or failed to reach the growing points of the grafted plant.

The variation in symptoms occurring in tobacco plants grafted with cions from recovered plants could not be accounted for on the basis of variation in plants or plant position from which cions were taken, of interaction of virus strains, of age of test plants at time of infection, or of growth rate of inoculated plants. There was but little correlation between severity and time after grafting when the symptoms first appeared.

The severity of symptoms induced in Turkish tobacco plants by sugar-beet curly-top virus after infection by means of the insect vector was dependent in part upon the portion of the plant on which the vector fed. Severe symptoms were invariably produced when the vector fed on tissues near the growing point, but frequently only mild or moderate symptoms resulted when the insect was confined to single leaves or portions of stem well below the growing point. Transmission of the virus to old tissues by the insect vector was thus comparable to transmission by grafting with cions from

recently diseased or recovered tobacco plants. These results leave doubt that any protective substances are transmitted through the graft union.

DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY,
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

## LITERATURE CITED

1. BENNETT, C. W. Plant tissue relations of the sugar-beet curly-top virus. Jour. Agr. Res. [U. S.] 48: 665–701. 1934.
2. ———. Correlation between movement of the curly top virus and translocation of food in tobacco and sugar beet. Jour. Agr. Res. [U. S.] 54: 479–502. 1937.
3. ———, and KATHERINE ESAU. Further studies on the relation of the curly top virus to plant tissues. Jour. Agr. Res. [U. S.] 53: 595–620. 1936.
4. CARSNER, E., and C. F. STAHL. Studies on curly-top disease of the sugar beet. Jour. Agr. Res. [U. S.] 28: 297–320. 1924.
5. KING, L. S. Studies in Eastern equine encephalomyelitis. IV. Infection in the mouse with fresh and fixed virus. Jour. Exp. Med. 71: 95–106. 1940.
6. KUNKEL, L. O. Studies on aster yellows. Am. Jour. Bot. 13: 646–705. 1926.
7. LESLEY, J. W. The resistance of varieties and new dwarf races of tomato to curly top (Western yellows blight or yellows). Hilgardia 6: 27–44. 1931.
8. ———, and J. M. WALLACE. Acquired tolerance to curly top in the tomato. Phytopath. 28: 548–553. 1938.
9. PRICE, W. C. Virus concentration in relation to acquired immunity from tobacco ring spot. Phytopath. 26: 503–529. 1936.
10. ———. Acquired immunity from plant virus diseases. Quart. Rev. Biol. 15: 338–361. 1940.
11. RIVERS, T. M., and F. F. SCHWENTKER. Vaccination of monkeys and laboratory workers against psittacosis. Jour. Exp. Med. 60: 211–238. 1934.
12. SELLARDS, A. W. The behavior of the virus of yellow fever in monkeys and mice. Proc. Nat. Acad. Sci. 17: 239–343. 1931.
13. SEVERIN, H. H. P. Additional host plants of curly top. Hilgardia 3: 595–638. 1929.
14. SHAPOVALOV, M. A celluloid cell for inoculation of plants with insect vectors. Phytopath. 20: 681–683. 1930.
15. ———. Graft versus insect transmissions of curly top in tomatoes (tomato yellows). Phytopath. 25: 844–853. 1935.
16. TENBROECK, C., and M. H. MERRILL. A serological difference between eastern and western equine encephalomyelitis virus. Proc. Soc. Exp. Biol. and Med. 31: 217–220. 1933.
17. THEILER, M. Studies on the action of yellow fever virus in mice. Ann. Trop. Med. and Parasitol. 24: 249–272. 1930.
18. TRAUB, E. Persistence of lymphocytic choriomeningitis virus in immune animals and its relation to immunity. Jour. Exp. Med. 63: 847–861. 1936.
19. WALLACE, J. M. Recovery from and acquired tolerance of curly top in *Nicotiana tabacum*. Phytopath. 29: 743–749. 1939.
20. ———. Evidence of passive immunization of tobacco, *Nicotiana tabacum*, from the virus of curly top. Phytopath. 30: 673–679. 1940.
21. ———. Virus strains in relation to acquired immunity from curly top in tomato. (Abstract) Phytopath. 32: 18–19. 1942.

# COMPARISON OF CERTAIN MERCURY AND NON-METALLIC DUSTS FOR CORN SEED TREATMENT[1]

P. E. HOPPE[2]

(Accepted for publication October 7, 1942)

The limitations on the civilian use of mercury necessitates an evaluation of non-metallic seed treatment dusts as possible substitutes for the mercury compounds in common use. The writer reports herein the results of laboratory and field experiments comparing the relative effectiveness on corn of the following seed disinfectants: Spergon (tetrachloro-para-benzoquinone), Thiosan,[3] (50 per cent tetramethyl thiuramdisulphide), New Improved Semesan Jr. (ethyl mercury phosphate, 1 per cent), and Barbak D (mercuric phenyl cyanamide, not less than 6 per cent). Barbak D differs from Barbak C (the corn disinfectant heretofore put out by the American Cyanamid and Chemical Corporation) in that it contains 2 per cent less of the mercury compound than the latter.

## LABORATORY EXPERIMENT

This experiment was designed to compare the seed disinfectants with respect to their fungicidal effects on *Diplodia zeae* (Schw.) Lév., one of the commonest and most virulent of the corn seedling blight fungi. In these tests diplodia-rotted kernels were surface-sterilized by immersing for 10 minutes in 0.46 per cent sodium hypochlorite solution, were dried and then dusted by dipping the kernels while held in forceps into the seed disinfectant. Excess dust was removed from the kernels. The kernels then were plated on slightly acidified potato dextrose agar in Petri dishes, one kernel per plate. The plates were kept at room temperatures and observations were recorded on the inhibitory effects of the various dusts as indicated by the amount and rate of growth of *D. zeae* from the corn kernels.

## RESULTS

All dust treatments retarded growth of the fungus and some of them often inhibited completely the growth of the fungus in the plates. Ranked in the order of their efficiency in retarding or inhibiting the fungus, the dusts were, New Improved Semesan Jr., Thiosan, Barbak D, and Spergon. The differences between the first 3 dusts mentioned were slight but Spergon was definitely inferior (Fig. 1). The experiment was repeated many times with practically identical results. In all these tests Spergon invariably was

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

[2] Associate Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] In experiments conducted since this manuscript was submitted, the writer has found the Dubay nonmetallic products, Thiosan and Arasan, essentially the same as to fungicide value and in ability to control *Diplodia* and *Gibberella* in seedborne infections.

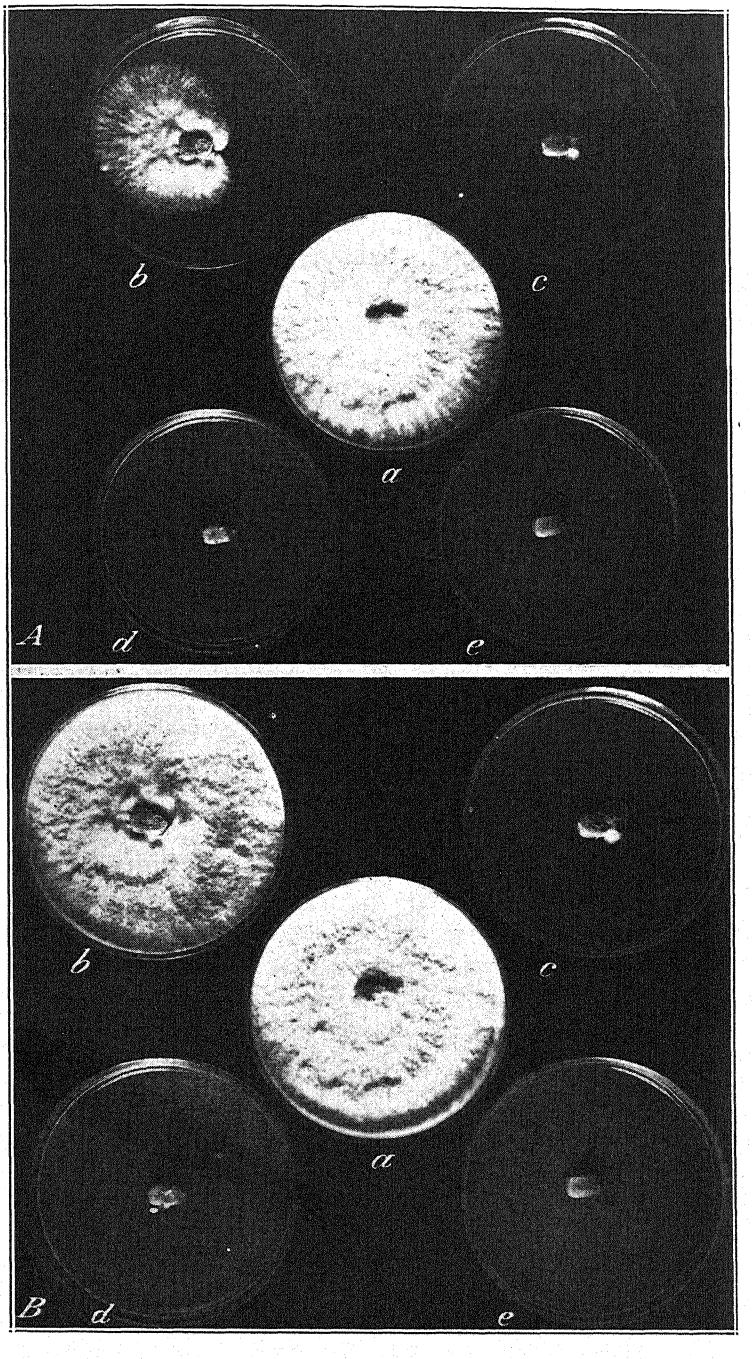



FIG. 1. Growth of *Diplodia zeae* from infected corn kernels given various seed treatments. *A*, 5 days after plating. *B*, Same plates 8 days after plating. The kernels were surface sterilized and then treated with the following seed disinfectants: *a*, control, not treated; *b*, *c*, *d*, *e*, treated with Spergon, Barbak D, Thiosan, and New Improved Semesan Jr., respectively.

conspicuous in its relative inability to inhibit the growth of *Diplodia zeae* from the rotted kernels.

A majority of the kernels in all the treatments eventually developed *Diplodia zeae*. The differences between treatments were mainly in degree of retardation. Throughout the experiment, which continued 10 days, the percentage of kernels that remained free from the fungus was higher in those treated with New Improved Semesan Jr. than in those treated with any of the other dusts.

## FIELD EXPERIMENT

Both diplodia-infected hybrid seed and nearly-disease-free inbred seed of dent corn were used in a field experiment. Unfortunately, the Bayer-Semesan Company's non-mercury product, "DuBay" 1205–FF (Thiosan), was not included in the field trial since the writer had none in his possession at the time of planting.

A limited amount of seed that was 100 per cent infected with *Diplodia zeae*, with the degree of infection so restricted that the kernels were germin-

TABLE 1.—*Stands from diplodia-infected and from nearly disease-free seed lots of corn following seed treatment with Spergon, Barbak D, and New Improved Semesan Jr.*

| Type of seed | Seed treated with | | | |
|---|---|---|---|---|
| | Control not treated | Spergon | Barbak D | New Imp. Semesan |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| Diplodia-infected hybrid | 24.4 | 40.0 | 77.8 | 91.1 |
| 71 nearly disease-free inbreds | 79.8 | 85.4 | 82.1 | 84.5 |

able, provided excellent material with which to compare the dusts for ability to control seedling blight from seed-borne infection. This seed was obtained by a very careful selection of kernels from hybrid ears that had been artificially inoculated with the fungus the previous season too late for complete ear rot development. The limited amount of the diplodia-infected seed available permitted only 45 kernels for each of the three seed treatments and the control. Each of these lots was planted in a 5-foot row.

The nearly disease-free seed consisted of 71 inbred lines of corn, obtained, for the most part, from the seed stocks of the Wisconsin Agricultural Experiment Station. These 71 inbreds were each planted 50 kernels per 5-foot row, in 2 replications, making 100 kernels for each inbred and a grand total of 7100 kernels for the inbreds for each treatment and for the controls.

The experiment was planted April 18, 1942, at Madison, Wisconsin, almost a month earlier than the usual corn planting time for this location. Planting was followed by a week of abnormally warm weather after which temperatures dropped and conditions became more favorable for seedling blight development. On the whole, conditions were not so favorable for the disease as might be expected some years.

RESULTS

Final notes were taken the first week in June when most of the seedlings had passed the 4-leaf stage of development. The data for stands for both the diplodia-infected and the nearly disease-free seed are given in table 1.

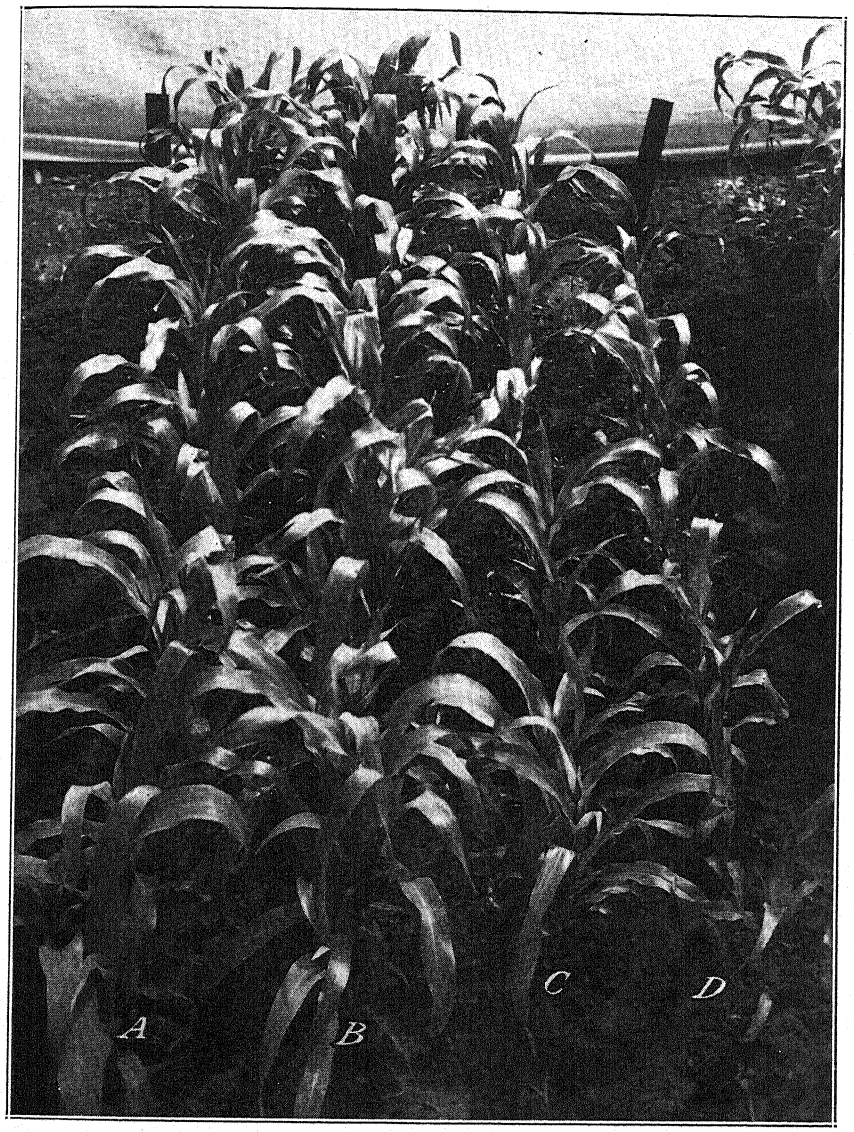



FIG. 2. Differences in stand and vigor of corn seedlings following seed treatment of diplodia-infected seed with different dust disinfectants. *A*, Barbak D. *B*, New Improved Semesan Jr. *C*, Spergon. *D*, Control. (Data in Table 1.)

Spergon nearly doubled the stand of the diplodia-infected control, but proved very inferior to the mercury dusts in controlling seed-borne infec-

tion. The differences between Spergon and the mercury dusts were actually greater than is indicated by the differences in stand. Most of the plants in the Spergon treated row were stunted and were found to have badly diseased mesocotyls. The plants in the rows treated with Barbak and New Improved Semesan Jr. were larger and relatively free from disease (Fig. 2).

All treatments of the nearly disease-free seed resulted in slight increases in stand, but the differences among the three kinds of dusts are small and of doubtful significance. Spergon appears to have been as effective as the mercury dusts in protecting the seed from soil infection.

## DISCUSSION OF RESULTS

One cannot generalize broadly on the subject of seed treatments from the results of experiments as limited in scope as those described above. The data and observations are reported in the hope they will be useful in supplementing the results of experiments by other workers.

The results obtained indicate promise for non-metallic dusts as possible substitutes for the mercury compounds. Those with Spergon indicate limitations in its usefulness in controlling seed-borne infection. It may have value, however, as a protectant dust for sound seed planted under unfavorable conditions. As was mentioned previously, it is very unfortunate that the Bayer-Semesan Company's non-metallic dust, "DuBay" 1205–FF (Thiosan), was not included in the field experiment. This dust was far superior to Spergon and about equal to the mercury dusts in the laboratory tests in inhibiting *Diplodia zeae*.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

# PYTHIUM ULTIMUM AND THE DAMPING-OFF OF COTTON SEEDLINGS[1]

C. H. ARNDT

(Accepted for publication October 3, 1942)

*Pythium ultimum* Trow[2] has been repeatedly isolated from diseased cotton seedlings in a field of well-drained, light sandy-loam soil at Clemson, South Carolina. This fungus also has been isolated from a small number of diseased seedlings in other areas during periods of cool, rainy weather by the writer and by Weindling, *et al.* (5). These observations on the association of weather conditions with the isolation of this fungus suggested the study of the relation of soil temperature to infection by this fungus as reported in this paper.

## METHODS

Cultures of the fungus were obtained from seedlings with small lesions by washing them with sterile water and then placing the diseased portion of the hypocotyls on raisin-oatmeal agar, adjusted to pH 5 with $KH_2PO_4$. Bacteria were eliminated from the cultures by making several successive hyphal-tip transfers from rapidly growing mycelia. Three isolates were used in the experiments. As they all showed a similar pathogenicity, no distinction between them will be made in the experimental data.

The effect of soil temperature on the infection of cotton seedlings by this fungus was determined by growing the seedlings at temperatures of 18, 21, 24, 27, and 30 degrees C. in soil-temperature tanks of the University of Wisconsin type. In each of the $25 \times 50$ cm. metal cylinders were placed 9 kg. of a steamed, light sandy-loam soil, which had a water-holding capacity of 36 per cent. A holard of 60 per cent was maintained by adding water as needed. Before planting, a one-eighth sector of 4-day-old culture of the fungus grown in a 9-cm. Petri dish on raisin-oatmeal agar was mixed into the upper portion of the soil of each cylinder. A like amount of sterile culture medium was added similarly to the cylinders in which the controls were grown.

Acid-delinted seed of a strain of Cleveland Big Boll cotton (Marett's 5–5) were used. To assure that only viable, disease-free seedlings were used, the seeds were immersed for 2 minutes in $\frac{1}{2}$ per cent $HgCl_2$ in 50 per cent ethanol, then rinsed with sterile water, and germinated at 30° C. on sterilized, moistened filter paper in Petri dishes, until the radicles had emerged 2–3 cm. The germinated seeds were placed, radicle end down, in 21 regularly spaced holes, $1 \times 4$ cm., that had been punched into the soil. The holes were then filled with soil and the surface was covered with 2 medium-weight discs of asphalt-impregnated roofing paper to facilitate

[1] Technical contribution no. 88 from the South Carolina Agricultural Experiment Station.

[2] Acknowledgment is made to Dr. Charles Drechsler for the verification of this determination.

maintenance of constant soil moisture and temperature. The discs were removed from the cylinders as soon as the cotyledons began to emerge, which varied with the temperature from 40 to 160 hours (Table 1) after planting. After the removal of the paper discs, the soil temperature at a depth of 2 cm. did not vary more than $\frac{1}{2}°$ from the mean temperature. Nearer the surface the variation was greater depending upon the differences between the soil and air temperatures and the intensity of the sunlight. All percentages in the tables, unless otherwise noted, are based on the planting of 21 seeds in each of 6 cylinders. The weights of the tops, the roots having been cut off at the base of the hypocotyl, were used to determine the relative growth of the seedlings.

## EXPERIMENTAL RESULTS

The results for one experiment in which the plants were grown in the greenhouse from March 21 to April 24 are given in table 1. During this

TABLE 1.—*The effect of soil temperature on the emergence and damping-off of cotton seedlings in soil inoculated with Pythium ultimum*

| Soil temperature | Total emergence | | Seedlings killed first 14 days | Seedlings after 30 days | | |
|---|---|---|---|---|---|---|
| | Relative to controls | Time required | | Alive | Healthy | Mean weights relative to controls |
| °C. | *Per cent* | *Hours* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 30 | 97[a] | 40 | 6 | 94 | 80 | 97 |
| 27 | 87 | 44 | 45 | 55 | 10 | 56 |
| 24 | 77 | 60 | 60 | 40 | 10 | 55 |
| 21 | 68 | 110 | 83 | 0 | 0 | ..... |
| 18 | 38 | 160 | 90 | 0 | 0 | ..... |
| 15–30[b] | 70 | 90 | 65 | 63 | 0 | 78 |
| Greenhouse[c] | 50 | 160 | 85 | 30 | 0 | 31 |

[a] Temperature 30° for 12 hours during the day and 15° at night.
[b] Plants on greenhouse bench. Containers similar to those used in constant temperature tanks.
[c] Estimated from plants removed after 14 days. Few lesions evident on the 30th day.

period the daily minimum range of air temperatures was 10–18°, with a mean of 16°; and the daily maximum range 20–37°, with a mean of 30°. In this experiment at 30, 27, 24, 21, and 18 degrees C., the seedling emergences were, respectively, 3, 13, 23, 32, and 62 per cent less than in the controls at the same temperatures, in which the emergences ranged from 93 to 100 per cent. When the alternating periods of 12 hours each at 15° and 30° were used, the reduction in emergence, as shown in table 1, approximated that at a constant temperature of 21°; while in the cylinders on the greenhouse bench, the emergence was slightly lower than at 21°. Fourteen days after planting, the seedlings in the cylinders at 27° and 30° were reduced to 10 to prevent crowding. These seedlings were removed by random selection according to a prearranged plan to avoid a possible bias in the removal of diseased and healthy seedlings. There were lesions on 20 per cent of these seedlings grown at 30°, and on 90 per cent of those grown at 27°. No seed-

lings were removed from the cylinders at the lower temperatures, as the number and size of plants was not sufficient to cause crowding.

At 30° 6 per cent of the seedlings that emerged were killed within the first week; then, there was no further evidence of injury by the fungus. At this temperature 20 per cent of the hypocotyls of the seedlings removed at the end of 14 days showed small superficial lesions. The injury was not sufficient to cause a stunting similar to that occurring at all lower temperatures. The degree of stunting of the seedlings is indicated in table 1 by the relative green weights of the seedlings in the controls and those in the inoculated cylinders.

At 27° and the lower temperatures the fungus invaded the stele of the hypocotyl and infected the roots, in contrast to the superficial lesions produced at 30°. All killing of the seedlings at 24° and 27° occurred within the first 14 days after planting, and all plants surviving after that period

TABLE 2.—*Injury to cotton seedlings by Pythium ultimum at soil temperatures of 22°, 27°, 30°; and also at 22° after initial growth periods at 30°*

| Soil temperature for successive growth periods | | | Relative green weight of seedlings grown in non-inoculated soil (controls) | Weight of seedlings in inoculated soil relative to seedlings in controls | Percentage of plants | |
|---|---|---|---|---|---|---|
| Days | | | | | With lesions | Killed |
| 1–6 | 7–12 | 13–19 | | | | |
| °C. | °C. | °C. | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 30 | 30 | 30 | 100 | 97 | 30 | 1 |
| 30 | 30 | 22 | 97 | 81 | 40 | 1 |
| 30 | 22 | 22 | 94 | 65 | 65 | 5 |
| 22 | 22 | 22 | 65 | 20 | 100 | 80 |
| 27 | 27 | 27 | 100 | 63 | 100 | 16 |

were growing normally at the end of the experiment. Only 17 and 10 per cent, respectively, of the plants grown at 21° and 18° were alive after 14 days, and all of these were dead at 30 days after planting. At all temperatures the first evidence of severe injury was a wilting of the seedlings, followed by their death within 2 or 3 days.

After determining the relative pathogenicity of the fungus at the several temperatures, seedlings were grown for 6- and 12-day periods at 30° in inoculated soil and then transferred to 22° to study the interaction of the age of the seedling and soil temperature on infection by this fungus. Three other sets of plants were grown throughout the 19-day period at 22, 27, and 30 degrees C. The atmospheric conditions during the growth of this series were much the same as in preceding series, except for a 3° higher mean minimum temperature. The percentages of seedlings infected and killed at the constant temperatures of 22, 27, and 30 degrees, table 2, were approximately the same as in the first series. In the inoculated soil a 10 per cent greater number of the seedlings grown for an initial period of 12 days at 30° and then transferred to 22° were infected than of those grown continuously at 30°. There was also a 16 per cent greater reduction in the mean weight of

the seedlings. The transfer of the seedlings to 22° after 6 days at 30° caused a further increase of 25 per cent in the number of infected seedlings, and, as might be expected from the lower relative weight, many of the seedlings were stunted. The stunted seedlings were making satisfactory growth on the 19th day and appeared capable of outgrowing the lesions and developing into normal plants. Only 5 per cent of seedlings grown for 6 days at 30° and then lowered to 22° were killed as compared to 80 per cent of those grown continuously at 22°.

In these experiments *Pythium ultimum* produced a soft rot of the cortex of the hypocotyl below the surface of the soil. The affected area was almost colorless to light-brown until the decay of the tissues had become well advanced, when they became darker. The lesions in their initial development tended to involve the entire circumference of the hypocotyl. In this respect they are similar to those produced by *Rhizoctonia solani* and differ from those produced by *Colletotrichum gossypii,* which first appear as elongated sunken areas in the cortex of the hypocotyl (5). Although the hyphae of *P. ultimum* were at first confined to the cortex, at 27° and the lower temperatures, the hyphae invaded the stele causing the wilting and death of the seedlings. This fungus did not seem to attack the roots until after the hypocotyl had been well decayed. In the experiment in which the plants were grown for 30 days, all of the seedlings alive at the end of this growth period were developing normal tissue underneath the lesions and were apparently capable of developing into normal plants. The rapid recovery of the seedlings from lesions produced by this fungus seems to indicate that it is strictly a seedling parasite of cotton. This is also indicated by the fact that no evident stunting of the surviving older plants has been observed in the area of the field in which the fungus had caused severe damping-off. Apparently it is a more transient parasite on cotton than related *Pythium* spp., which parasitize corn (3) and sugar cane (4).

Harter and Whitney (2) have shown that *Pythium ultimum* grows equally well over a temperature range of 23–35° C., the rate of mycelial spread on agar falling off rapidly above 35° and somewhat less so below 23°. Consequently, if the rate of mycelial growth were the only factor involved, the most severe injury to cotton seedlings should have occurred in these experiments at 30° instead of at temperatures below 27°. Similarly, Harter and Whitney (2) found no direct correlation between the relative rate of growth of the fungus at various temperatures and its injury to the sweet potato at the same temperatures. Much the same situation has been found for the *Pythium* species that parasitize the roots of sugar cane and corn. Flor (1) and Rands and Dopp (4) found that the optimum for the growth of these mycelia was close to 30°. Yet Flor (1) found that the damage to corn was greater at lower temperatures and at a high soil moisture content. Injury to corn at 30° was, however, relatively greater than that to cotton seedlings caused by *P. ultimum.*

The widespread distribution and diversity of the hosts of *Pythium ultimum* would seem to indicate that it may be a frequent, although probably

not an economically important, cause of the damping-off of cotton seedlings. Because of the well-known possibility of inhibiting the growth of *Pythium* spp. by most of the fungicides used for the surface sterilization of cotton seedlings before they have been plated on water agar for the determination of infecting fungi (5), it is not unlikely that a different technique might result in a more frequent recovery of this fungus from diseased seedlings.

## SUMMARY

*Pythium ultimum* has been found to be a frequent cause of the damping-off of cotton seedlings in one field in South Carolina when planting has been followed by cool, rainy weather.

When cotton seedlings were grown at soil temperatures of 18, 21, 24, 27, and 30 degrees C. and a soil-moisture content of 60 per cent, this fungus caused little damping-off at 30°, but caused severe damping-off at the lower temperatures. All seedlings were killed at 21° and 18°. The results obtained when the seedlings were grown for 6 days and 12 days at 30° in inoculated soil and were then transferred to 22° indicate that this fungus will cause severe losses from damping-off only if soil conditions are favorable for infection before the seedlings have reached a stage of development comparable to that reached in a growth period of 6 days at 30°.

## LITERATURE CITED

1. FLOR, H. H. Relation of environmental factors to growth and pathogenicity of Pythium isolated from roots of sugar cane. Phytopath. 20: 319–328. 1930.
2. HARTER, L. L., and W. A. WHITNEY. Mottle necrosis of sweet potatoes. Jour. Agr. Res. [U. S.] 34: 893–914. 1927.
3. JOHANN, H., J. R. HOLBERT, and J. G. DICKSON. A Pythium seedling blight and root rot of dent corn. Jour. Agr. Res. [U. S.] 37: 443–464. 1928.
4. RANDS, R. D., and E. E. DOPP. Variability in *Pythium arrhenomanes* in relation to root rot of sugar cane and corn. Jour. Agr. Res. [U. S.] 49: 189–221. 1934.
5. WEINDLING, R., P. R. MILLER, and A. J. ULLSTRUP. Fungi associated with diseases of cotton seedlings and bolls, with special consideration of *Glomerella gossypii*. Phytopath. 31: 158–167. 1941.

## PHYTOPATHOLOGICAL NOTES

*Freezing Preservation of Fungi and Fungus Spores.*—The sporidia of *Gymnosporangium juniperi-virginianae* and the conidia of *Venturia inaequalis* have been used as test fungi in the method for evaluating fungicides for the control of apple scab and cedar-apple rust on potted trees in the greenhouse. Difficulties were encountered in maturing the cedar galls for early season work and keeping them throughout the year after spore discharge was complete in the field. As it was impractical to culture conidia of *Venturia inaequalis* in sufficient quantity for the experiments, the earlier investigations with this fungus were limited to unreliable sources of inoculum from the field. A new technique involving quick-freezing and storage of the galls and conidia at low temperatures has been found to provide a readily

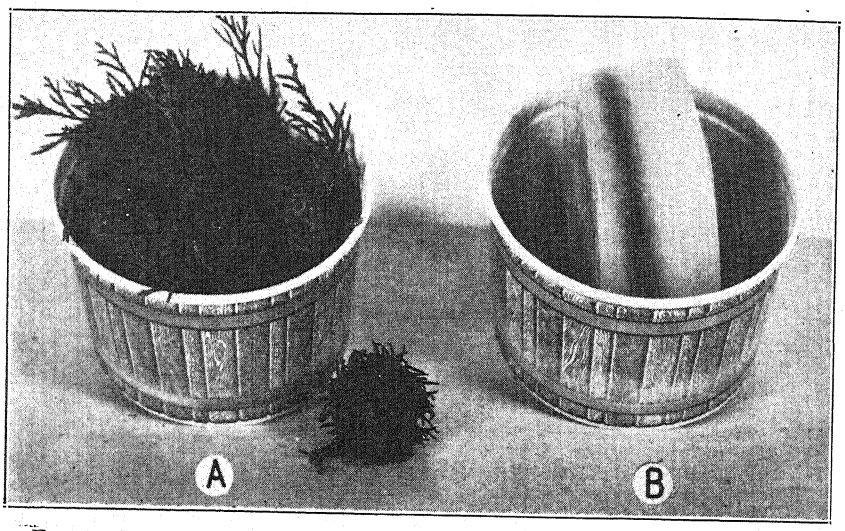



FIG. 1. Fungus material as frozen and stored at −10° C. A. Cedar-apple galls. B. Conidia of *Venturia inaequalis.*

available source of inoculum throughout the year (Fig. 1). It is thought that this procedure would be of interest to other workers interested in the storage of fungi.

### STORAGE OF GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE

Cedar galls, three-quarters of an inch and over in diameter, were selected with telial horns approximately one-half inch long when dry, placed in paraffined Dixie cups, and held at a temperature of −10° C. Excessive dehydration over a prolonged period is prevented by placing the cups in vapor-proof metal containers. The galls are removed from cold storage to room temperature about 12 hours prior to placing them in a misty spray in preparation for spore discharge. The germination of teliospores is not impaired by the freezing process. Galls have been first frozen at −40° C., for a short time and then raised to −10° C., but this is not considered essential.

Foliage tests have shown sporidia obtained from the frozen galls as viable as those obtained from galls taken from the field. The teliospores on galls held at −10° C. for 9 months have functioned normally. It is of special interest that three spore discharges have been obtained from a given gall that had been refrozen after each discharge. Viable sporidia have been obtained 15 months after the first freezing of such galls. Sporidia in water suspension have been stored at −10° C. and −40° C., but cannot be held longer than 3 weeks without appreciable loss in viability.

### STORAGE OF VENTURIA INAEQUALIS

Concentrated aqueous suspensions of conidia of *Venturia inaequalis*, washed from fresh fruiting lesions on foliage or fruit, are stored at −10° C. in paraffined Dixie cups. To prevent the spores settling to the bottom of the cups where they would be subjected to dehydration and consequent loss of viability during storage, it is necessary to prepare the cups with a layer of ice frozen in the bottom before the spore suspension is poured. The inoculum is obtained by chipping a portion of ice from the block, defrosting, and diluting to the desired concentration.

Excellent germination of conidia frozen more than 15 months has been obtained. While the frozen conidia may be satisfactory for routine testing on glass slides or on the foliage, they are more readily inhibited by fungicides than fresh-borne conidia. In other words, the slope of the LD–50 line becomes steeper after the conidia have been stored for an appreciable length of time.

The spore suspensions have been frozen at −40° C. without injury.

This procedure has worked equally well with the conidia of *Sclerotinia fructicola*. Dr. R. F. Suit has had comparable results from freezing the conidia of *Plasmopara viticola*.—J. M. HAMILTON and L. O. WEAVER, N. Y. State Agricultural Experiment Station, Geneva, New York

*"Red Ring" of Tomato Stems Caused by an Insect, Cyrtopeltis varians (Dist.), at Charleston, S. C.*[1]—During August, 1941, a mirid, *Cyrtopeltis varians* (Dist.),[2] (Fig. 1, C, D), formerly named *Engytatus geniculatus* Reuter, was observed associated with reddish-brown marks (Fig. 1, A) encircling the stems and petioles of tomato plants. This condition was observed again from July to October, 1942, at the Regional Vegetable Breeding Laboratory of the U. S. Department of Agriculture at Charleston, South Carolina. The injury occurred on the upper and youngest branches of the plants, and was most evident late in the picking season. It was observed throughout the plots, occurring on such varieties as Rutgers, Marglobe, Pan America, and Stokesdale.

[1] Contribution No. 28, U. S. Regional Vegetable Breeding Laboratory, Charleston, South Carolina.

[2] Specimens from both 1941 and 1942 collections (E. and P. Q. No. T. C. 3968) were determined by H. G. Barber, Division of Insect Identification, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Washington, D. C.

The insect had girdled the stems with punctures approximately 1 mm. deep. Soon after the injury, the marks were barely discernible; later they turned reddish-brown and were usually raised above the normal surface of the stems. Bending the stems or petioles often caused breakage (Fig. 1, B).

This plant bug occurs throughout southern United States to Lower California, Mexico, Central America, Brazil, the West Indies, and the Hawaiian Islands. It has been reported[3] as a tomato pest in Texas and Arizona, where the injuries resembled closely those observed at Charleston. In the former States it was noted also that the stems with younger injuries broke off readily, whereas those with older injuries were quite resistant. This was

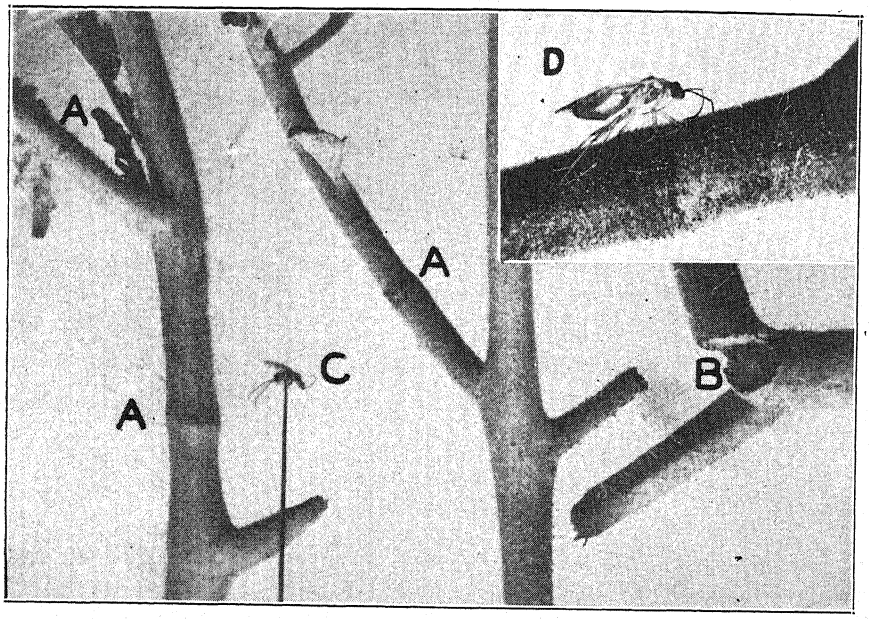



FIG. 1. "Red ring" injury to tomato stems. A. Rings on stems and leaf petioles. B. Stem broken at point of injury. C and D. Insect causing the injury. Note (insert) proboscis extending back from lower part of head. A to C, ×1.5. D, ×5.

also evident at Charleston, suggesting that healing of the wounds strengthened the stems at the points of injury. The insect has been reported[4] as a tomato pest in Georgia and Mississippi, but no descriptions of the injuries were given.

In the Hawaiian Islands the insect may cause tomato-crop failures by sucking the juice from the developing ovaries.[5] It has been observed on the islands of Maui,[6] Oahu,[7] and Kaui[8] in addition to Hawaii. No injuries

[3] Jones, W. W. Tomato vines injured by a Mirid (*Engytatus geniculatus* Reuter). J. Econ. Ent. 24: 327–328. 1931.

[4] Knight, H. H. The plant bugs, or Miridae, of Illinois. Ill. Nat. Hist. Surv. Bull. 22 (1): 53. 1941.

[5] Illingworth, J. F. *Engytatus geniculatus* Reuter—an important pest of tomatoes in Hawaii. Hawaiian Ent. Soc. Proc. 7 (2): 247–248. 1929.

[6] Hawaiian Ent. Soc. Proc. 7 (1): 2. 1928.

[7] *Ibid.*, 7 (1): 11. 1928.

[8] *Ibid.*, 7 (2): 271. 1929.

resembling "red ring" were described in these reports, the principal injuries being to the flower parts.

The Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, has cited an unpublished note made in 1940 by R. E. Campbell and J. Wilcox, located at its Alhambra, California, field laboratory as follows:

> A small bug, *Cyrtopeltis varians* (Dist.) is causing serious damage to tomatoes in Orange County. The immature bugs cluster on the twigs ¼ inch in diameter and less, and their feeding causes a brownish ring which girdles the twig and it usually breaks off.

W. H. White of the above-mentioned Division has made the following comment:

> In a survey performed by Messrs. Campbell and Wilcox it was found that this mirid bug was very numerous in Orange, Los Angeles, and San Diego counties in southern California during 1940. It was generally believed by the growers that the insect was responsible for the poor setting of tomatoes. Although signs of feeding injury of this insect were evident and in many fields the blossoms and newly-set tomatoes had dropped from the plants in large numbers, no definite information was obtained respecting the direct responsibility for the poor setting of the fruit. There was, however, a very evident relation between the number of these insects in a given field and the number of tomato fruits which set.

No serious injury of tomato plants was evident at Charleston, perhaps because the insects attacked only stems and leaves instead of flowers. The fact that the "red rings" might be mistaken for disease symptoms and that the insects might be vectors for some transmissible disease suggested this note on their occurrence.—GEORGE B. REYNARD, Assistant Geneticist, Div. Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Charleston, S. C.

*Southern Blight, Corticium rolfsii, of Potato Tubers.*—Potato tubers, variety Sebago, infected with *Corticium rolfsii* (Sacc.) Curzi, were observed when harvested in late May, 1942, in a field near Gainesville, Florida. In this field about 1 per cent loss of tubers was recorded.

Infections caused by this fungus on potato plant stems have been observed annually in the Southeastern States and described from Florida by the writer.[1]

Other records[2,3,4,5,6] concerning this disease include various descriptions of infected tubers and tops. Eddins[7] observed the disease in Florida and

[1] Weber, George F. Potato diseases and insects. Fla. Agr. Exp. Stat. Bull. 169: 123–125. 1923.

[2] Edson, H. A., and M. Shapovalov. Parasitism of *Sclerotium rolfsii* on Irish potatoes. Jour. Agr. Res. [U. S.] 23: 41–46. 1923.

[3] Dykstra, T. P. Potato diseases and their control. U. S. Dept. Agr., Farmers' Bull. 1881. 15–16. 1941.

[4] Higgins, B. B. Physiology and parasitism of *Sclerotium rolfsii* Sacc. Phytopath. 17: 417–448. 1927.

[5] Link, G. K. K., and G. B. Ramsey. Market diseases of fruits and vegetables, potatoes. U. S. Dept. Agr. Misc. Pub. 98: 46–48. 1932.

[6] McClintock, J. A. A tuber rot of Irish potatoes. Tenn. Agr. Exp. Stat. Circ. 32: 1930.

[7] Eddins, A. H. Diseases of plants in the United States. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. 25: 354. 1941. Suppl. 119: 241. 1939; Suppl. 128: 290. 1940.

recorded resultant losses. The available information describing this tuber disease is so variable and incomplete that a detailed description should be useful.

Suspected tubers, incubated for 24 hours at room temperature, bore the characteristic mycelium of the fungus. Isolated diseased tissue on potato-

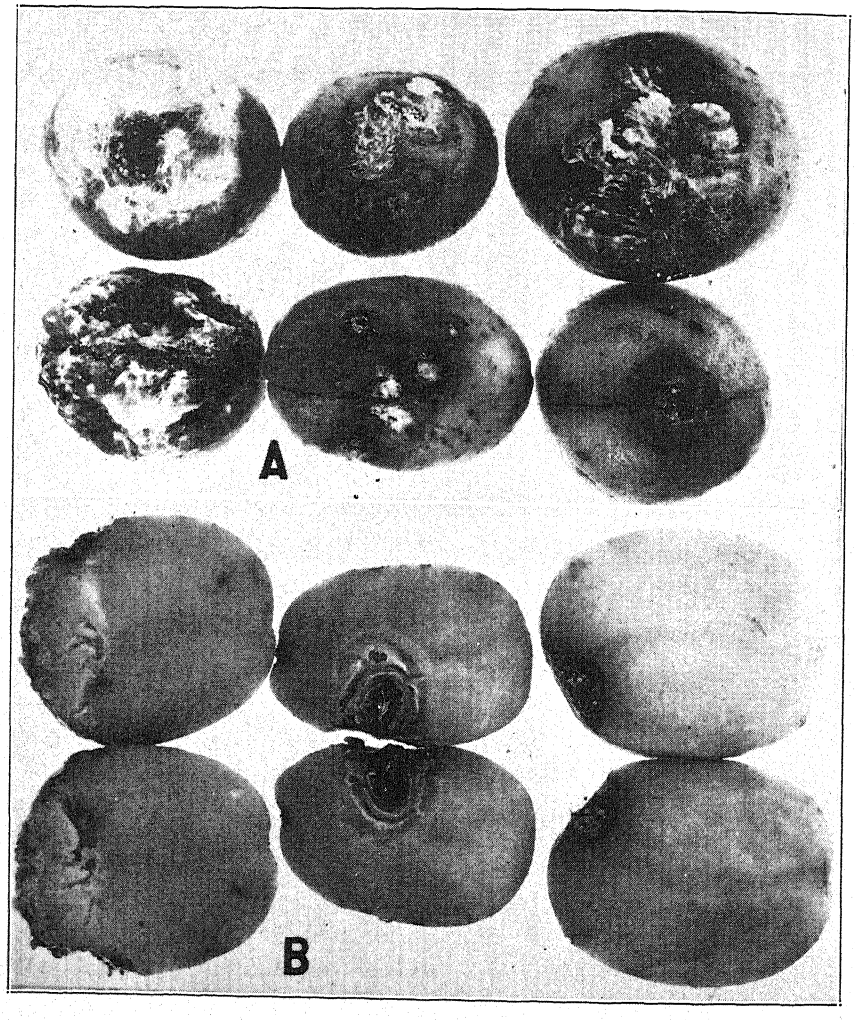



FIG. 1. Potato tubers infected with *Corticium rolfsii*. A. Various stages of development of the disease; note young sclerotia in upper left and center. B. Tubers of second row of A cut and showing internal decay.

dextrose-agar plates characteristically produced the fungus. Mycelium placed in contact with uninjured tubers, and mature sclerotia inserted into tuber wounds produced disease similar to that found in nature. The following symptoms generally characterize the disease (Fig. 1).

Externally the smallest characteristic infection spots were 2 to 3 mm. wide, circular, slightly sunken with brownish margin diffused into surrounding tissue. Area within the margin abruptly paler, with a center speck, apparently a lenticel. These spots isolated, widely scattered or clustered, but not correlated with any part of tubers. As spots enlarged, surface became more noticeably sunken, uniform yellow to tan. Spots about 1 cm. in diameter, gradually darkened as secondary organisms developed on surface. Frequently, tuber cortex surrounding these spots overgrown by thin, more or less reticulate hyphal weft closely applied to surface. As disease advances, portions of the tuber collapse, producing soft areas, usually covered by cortex. Ultimate and extensive development of infection ends in rupture of cortex and usual disclosure of sclerotia, clustered, white to dark-brown, depending on maturity, uniformly spherical and small.

Internally, the small rapidly developing young spots were shallow. As they enlarged and deepened the tuber flesh changed from a wet, firm, more or less transparent condition to an odorless, chalky white, opaque, cheesy consistency, losing its firmness entirely. Invaded portion dried, shrinkage and collapse followed, forming cavities later filled with the white mycelum of the fungus. Sclerotia usually developed in these cavities.

The plot of dry, rather porous, sandy soil, where the diseased tubers were grown, had previously been devoted to vegetable crops and several of them had shown frequent infection from this fungus. Previous observations have shown soil moisture the most important environmental factor affecting the development of the disease. During humid, rainy weather the fungus attacks plants at the soil surface, producing stem girdling and infection on aerial plant parts in contact with the soil. As the soil surface becomes dry the fungus appears to cause infection at successively lower levels. Often plants wilt because of stem girdling when ½ to 1 in. dry mulch exists. In such instances the points of infection are an inch or more below rather than at the surface. Certain deep-set plants frequently cultivated and hilled up during extremely dry seasons have been attacked by the fungus on the main tap root more than 3 inches below the surface. This moisture relationship has been determined by repeated examinations and seasonal observations of *Corticium rolfsii* and its pathogenicity on various wild and cultivated plants in Florida for a score of years. The application of this information here explains, in a general way, the current attacks on potato tubers. The dry season has apparently resulted in the development of the fungus at a deeper level in the vicinity of the tubers, resulting in their infection.—GEORGE F. WEBER, Agricultural Experiment Station, University of Florida, Gainesville, Fla.

*A Culture Medium of Maize-meal and Starch Mush to Replace Agar for Growing Fungi.*—I have been using a culture medium to partly replace agar in some of my work that may be worth suggesting for trial by others. Supplies of agar are difficult to obtain and a mixture of maize meal and starch was developed to replace routine use of large quantities of agar for some studies. The meal and starch are cheap, obtainable from grocery stores, and not likely to be curtailed by military necessities. In addition the mush medium has produced unique results in certain cultural studies. I have also used it with success as an acidified medium to isolate fungi from infected tissues.

My formulae and methods of preparation are as follows:

1. White maize meal (water-ground), 260 g. or 300 g. if steel-cut; cold tap water, 1000 cc. Mix these, bring to boil, and cook in double boiler or Arnold steam sterilizer for 10 minutes.

2. Corn starch (refined cooking quality), 20 g.; cold tap water 50 cc. Stir together until smooth. Pour into the hot mush just prepared (1), stirring constantly.

3. With spoon, dip about 25 cc. of this final hot-mush mixture into bottoms of Petri dishes and spread quickly and evenly by circular movements with glass rod, "settling" the mush by 2 sharp jolts on the palm of the hand.

4. Filled dishes are then covered and put directly in an autoclave. Pressure is slowly raised to 15 lb., where it is kept for 20 minutes, and then cooled slowly. These plates are allowed to cool overnight before using for culture purposes.

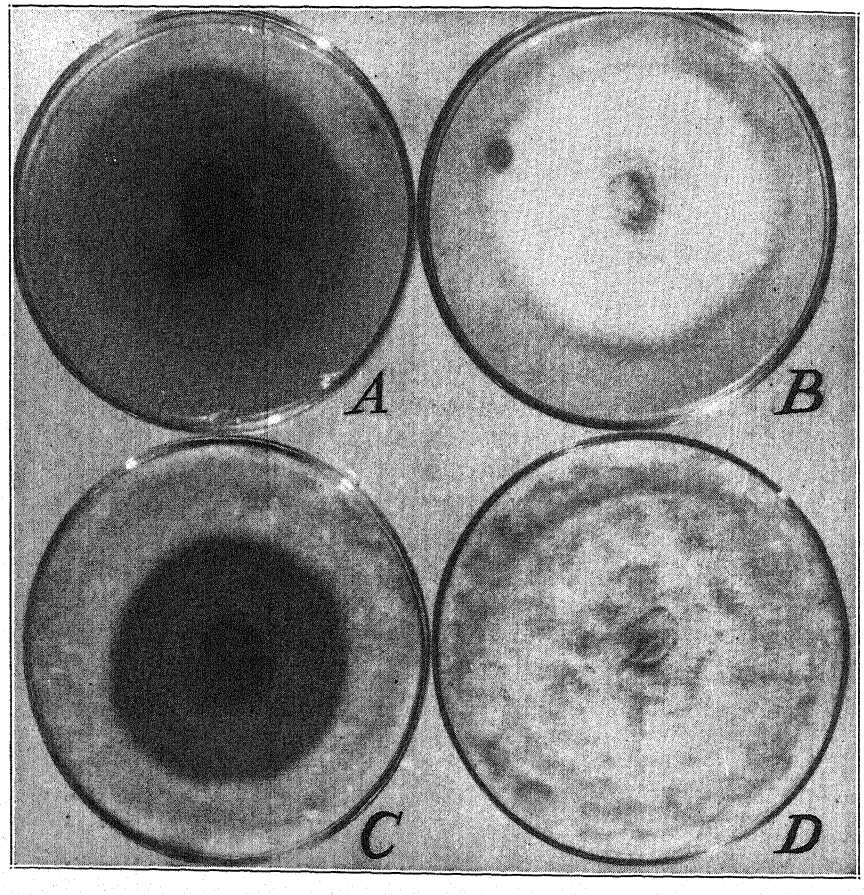



FIG. 1. Comparative growth in parallel cultures: *Alternaria solani*, on agar plate *A* and on maize-meal and starch-mush plate *C*; *Fusarium bulbigenum* var. *lycopersici* on agar plate *B* and on mush plate *D*. Agar is of "differential" formula (Wellman, F. L. Phytopath. 32: 271–287, 1942) known to produce excellent growth of both organisms. Mush-medium formula as given in present paper.

When acidified plates are to be prepared, 4 drops of 25% lactic acid are put on top of the mush before spreading and mixed in each dish at stage 3 of preparation.

Mush, after completing stage 2, may be thinned with about 10 per cent by volume of cold water. This thinner mixture can then be squeezed through a

large-bore funnel into test tubes, where it is slanted by jolting cautiously on the heel of the hand, plugged in the usual manner with cotton, autoclaved in a slanting position, and cooled in a slanting rack.

The mush medium is, of course, opaque, and has other disadvantages when compared with agar. It may not be so neatly prepared as some of the more transparent media. The operator can, however, become adept in its preparation and much culture work can be accomplished without benefit of agar.

Cultures of *Alternaria solani* (Ell. and Martin) Jones and Grout, and *Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R. grow very well on the mush medium (Fig. 1) and tests have shown that the cultures retained their pathogenicity. Other species of *Fusarium* and *Alternaria* and species of *Rhizoctonia, Colletotrichum, Penicillium, Aspergillus, Pythium,* as well as contaminating bacteria and yeasts, also have grown readily on the medium.—FREDERICK L. WELLMAN, formerly of Bureau of Plant Industry, now of Office of Foreign Agricultural Relations, U. S. Department of Agriculture, Washington, D. C.

*A Reflector Scale for Measuring Growth of Fungi.*[1]—Measurement of growth rate in certain microorganisms is a time-consuming process with

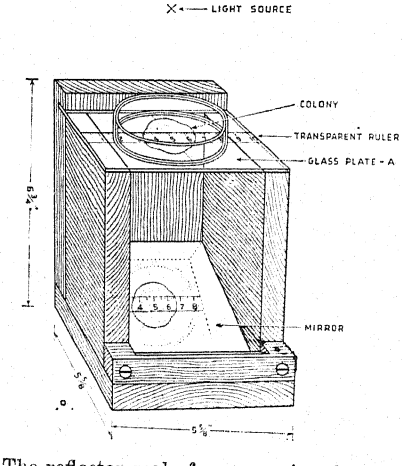



FIG. 1. The reflector scale for measuring fungus cultures.

danger of contamination and formation of secondary colonies when plates are disturbed for periodic measurements. Recently, in connection with a nutritional study of the effect of trace elements on various pathogens, several hundred agar plate cultures of various species of fungi were inoculated to study mycelial growth rate. For a reliable growth curve it was necessary to record the average diameter at 8-hour intervals from 2 measurements made at right angles. For convenience and speed of measurement, and to avoid

[1] Paper No. 2063 in the Scientific Journal Series, Minn. Agr. Exp. Station. Assistance in the preparation of figure 1 was furnished by personnel of the Works Project Administration official project No. 265–1–71–236, Subproject 487.

unnecessary disturbance and scattering of spores over the plate, the following simple apparatus was designed and built (Fig. 1). It is a compact, box-like instrument with a top panel of glass that is scaled in millimeters. A cheap, movable, transparent ruler, inserted just beneath the glass, is satisfactory if the glass is not ruled. A mirror fixed in the base of the frame at a 30° angle reflects whatever is placed on the glass. A desk lamp is placed above the apparatus, so that its light shines through the Petri plates placed right side up on the glass. The mirror image of the fungus culture on the agar is plainly visible and the scale is easily read. The light above the plate facilitates accurate measurements of colonies with extremely fine mycelium and indistinct margins.

The reflector scale may be used for fungus cultures in flasks as well as for cultures in Petri plates.—PHILIP HAMM, J. E. MITCHELL, and DAVID GOTTLIEB, Division of Plant Pathology and Botany, Minnesota Agricultural Experiment Station, University Farm, St. Paul, Minn.

*Rasp Leaf of Cherry.*—In May, 1940, C. W. Neider, State Horticultural Inspector for northern Idaho, sent to the writer a few diseased cherry leaves from a tree in the city of Coeur d'Alene. According to the owner the original sweet-cherry tree had suffered severe winter injury in 1935–36, and sprouts from the roots (mazzard) replaced the tree. No leaf abnormalities had been observed until 1940. The symptoms exhibited by leaves of this tree, and, subsequently, on experimental trees, gave rise to the term *ruffled leaf* by the writer, who at that time had seen no previous mention of this disease or its name in the literature. The characteristics of the disease in Idaho are regarded as identical with what Bodine and Newton designated as rasp leaf[1] and Lott and McLarty[2] called leaf enation. Symptoms are illustrated in figure 1. H. R. McLarty saw the Idaho trees in 1942 and stated that the symptoms apparently were the same as those shown by his material. The similarity in symptoms was noted also by L. C. Cochran who had seen the Colorado rasp leaf condition. In the interests of uniformity and priority the name rasp leaf is preferred.

Transmission trials started in August, 1940, showed by June, 1941, that in all but one or two cases, shoots produced by supposedly diseased buds did not even perpetuate the condition. On 2 mazzard seedlings, however, the stock showed mild symptoms; but, inasmuch as leaf enations may sometimes be found on mazzard seedlings, the proof of transmission was not convincing enough to warrant a statement on such a striking disease. In August, 1941, additional inoculation trials were made involving the use of young Bing trees, Montmorency, and additional mazzard seedling trees and two types of inoculum: (a) buds from the axils of affected leaves and (b) buds from

[1] Bodine, E. W., and J. H. Newton. The rasp leaf of cherry. Phytopath. 32: 333–335. 1942.

[2] Lott, T. B., and H. R. McLarty. Leaf enation. (*In* Handbook of virus diseases of stone fruits in North America, compiled by E. M. Hildebrand, G. H. Berkeley and D. Cation.) Mich. Agr. Exp. Stat. Misc. Pub. 51. 1942.

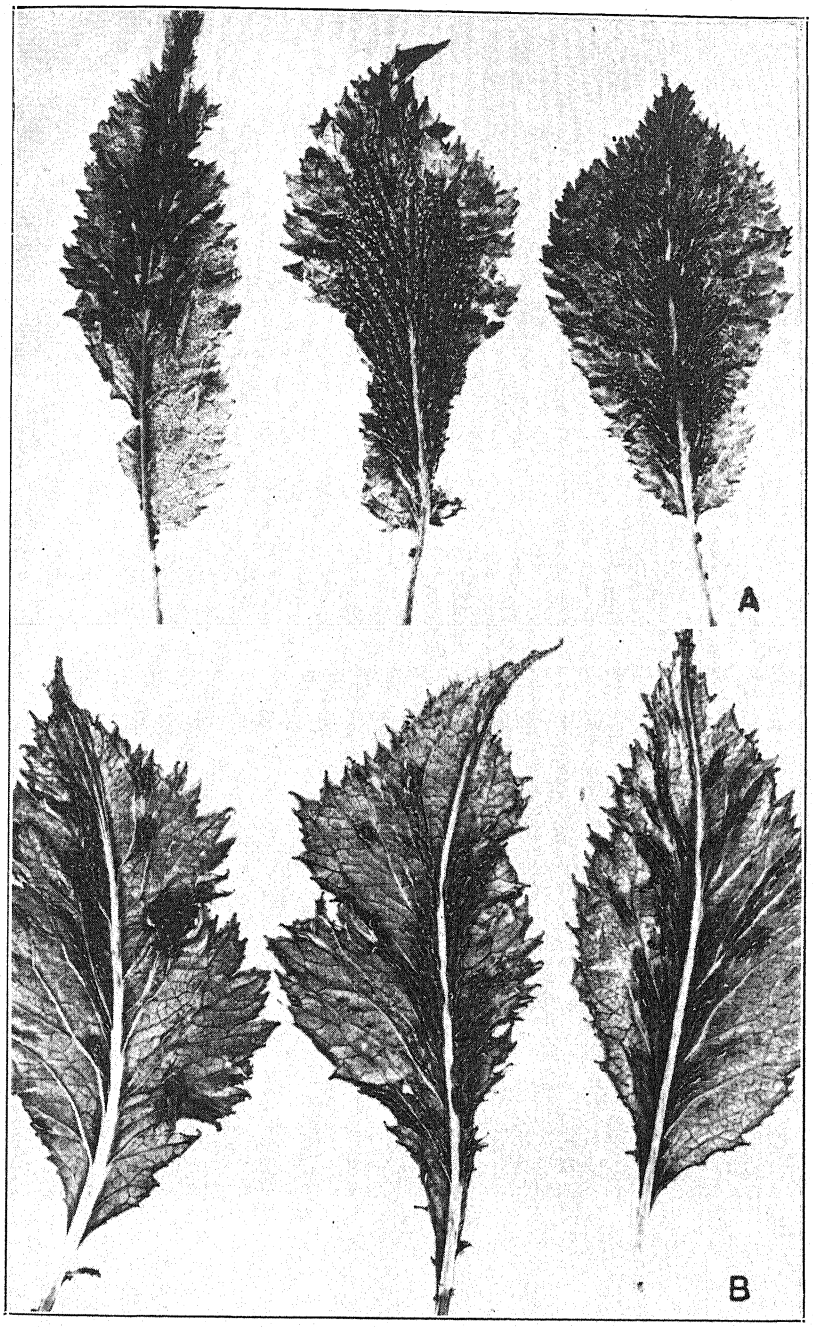



FIG. 1. Rasp leaf of cherry. A. Leaves from mazzard cherry seedling on which diseased buds had been placed. B. Leaves from Bing cherry on which diseased buds had been placed. The leaves on cion shoots showed even more distortion and leaf enations.

the axils of symptomless leaves borne on laterals of small branches that had some affected spurs.

By May, 1942, there was convincing proof in the nursery plots at Moscow that transmission of the virus had actually been accomplished in 1940–41, and symptoms were very pronounced in the second growing season. On Bing, on mazzard seedlings, and on Montmorency nursery trees inoculated in August, 1941, rasp-leaf symptoms showed on leaves growing near the point of insertion of diseased buds, although in some cases the buds had died during the winter. Trees inoculated with the (b) type bud wood showed no symptoms.

The writer's studies on rasp leaf of cherry in Idaho permit the following conclusions:

1. The rasp leaf of cherry, caused by a virus, is transmitted readily by bud inoculation; has an incubation period of less than 1 year (9 months) rather than 1 year (if spring grafted), as reported by Lott and McLarty[2] or 2 years, as reported by Bodine and Newton.[1]

2. Mazzard cherry seedlings, Bing cherry, and Montmorency cherry showed rather typical rasp-leaf symptoms 9 months after budding in August.

3. Observations supported by limited inoculation tests indicated that the rasp-leaf virus may be unevenly distributed in a host and probably moves rather slowly.

4. Rasp-leaf symptoms may be exhibited by leaves on terminal shoots of mazzard seedlings that have been bud-inoculated.

5. At present, rasp leaf is of no commercial importance in Idaho and no natural spread is known to have occurred. The devitalizing effect of rasp leaf, however, indicated the potential seriousness of the disease.

The widely separated areas in which rasp leaf has been reported—Colorado, British Columbia, and Idaho—raise the perplexing question of the origin and history of the virus.—EARLE C. BLODGETT, Idaho Agricultural Experiment Station, Moscow, Idaho.

# BOOK REVIEW

FOSTER, ADRIANCE S. *Practical Plant Anatomy.* 155 p. D. Van Nostrand Co., Inc., 250 Fourth Ave., New York. 1942. $2.50.

In this inexpensive little book the Plant Pathologist finds an up-to-date presentation of the essentials of the subject of Plant Anatomy that he is likely to need. The graduate student in Plant Pathology will likewise find this book helpful because it covers the fundamental aspects of the subject that he is expected to know, and does not require the study of a large volume of detail in order to obtain the desired information.

References covering the most important literature are listed at the end of each chapter. These provide a rapid means of locating classic as well as recent important contributions on various aspects of Plant Anatomy.—T. E. RAWLINS, Plant Pathology Division, University of California, Berkeley, California.

# DEFINITIONS OF FUNGICIDE TERMS[1, 2, 3]

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, COMMITTEE ON STANDARDIZATION OF FUNGICIDAL TESTS

TOXICITY: The ability of an agent to interfere adversely with the vital processes of an organism by physico-chemical means. TOXICANT: An agent capable of exhibiting toxicity; a poison.

FUNGICIDE: A chemical or physical agent that kills or inhibits the development of fungus spores or mycelium. A toxicant for fungi. BACTERICIDE: A toxicant for bacteria. INSECTICIDE: A toxicant for insects. NEMATOCIDE: A toxicant for nematodes.

PROTECTANT FUNGICIDE: An agent that protects the plant or plant part from infection by killing, or by inhibiting the development of fungus spores or mycelium that may arrive at the infection court (8, 14). In case of seeds—SEED PROTECTANT. PROTECTIVE VALUE: Measure of the ability of a protectant fungicide to prevent infection of the plant or plant part (7).

DISINFECTANT (general usage): An agent that kills or inactivates organisms on or within the plant or plant part, or the immediate environment. In case of seeds—SEED DISINFECTANT; soils—SOIL DISINFECTANT. A more precise and logical definition is to be preferred (1, 14), thus DISINFECTANT (restricted usage): An agent that frees from infection by destroying the pathogen established within the plant or plant part. Coordinate with this restricted use there is DISINFESTANT: An agent that kills or inactivates organisms present on the surface of the plant or plant part, or in the immediate environment. In the case of seeds—SEED DISINFESTANT; soils—SOIL DISINFESTANT.

ERADICANT FUNGICIDE: An agent applied to a plant, plant part or the environment to destroy fungi established within a given area of land or plant (8). Eradicant fungicides may function as disinfestants, disinfectants or both.

COLLOIDAL FUNGICIDE: A fungicide applied in the colloidal state, that is, made up of particles exhibiting Brownian movement which stay indefinitely dispersed and are within the range 0.2 to 0.005 $\mu$ in diameter (4).

FUMIGANT: A chemical toxicant employed in volatile form.

FUNGICIDAL (general usage): Pertaining to the ability of a fungicide to kill or inhibit the growth of fungi. FUNGICIDAL VALUE: Measure of ability of a fungicide to kill or inhibit the growth of fungi (7). FUNGICIDAL (restricted usage): Pertaining to the ability of a fungicide to kill fungi. In contrast (9) there is FUNGISTATIC: Pertaining to the ability of a fungicide to inhibit the germination of fungus spores or development of mycelium, while in continued contact.

PHYTOTOXIC: Pertaining to the property of killing or injuring plants, especially higher plants or their parts. Use in preference to PHYTOCIDAL.

[1] Copies may be obtained from the Committee Chairman, Boyce Thompson Institute, Yonkers, New York.

[2] Definitions from here on may be generalized by substituting toxicant for fungicide.

[3] In 1939 a Committee on the Standardization of Fungicidal Tests was appointed by The American Phytopathological Society. The general aims of the Committee are to improve the methods of testing fungicides in the laboratory and field through cooperation and standardization.

Cooperative research is sponsored on the evaluation of various experimental designs, on the development of new methods, and in the correlation of field and laboratory results. Fungicide is defined in its broadest sense, and the methods include any phase in the testing other than chemical analyses. The procedure in standardizing is as follows: A certain method is brought to the attention of the Committee as being desirable, that is, it fulfills a definite need and has given satisfaction in at least several different laboratories. A mimeographed outline of the method is prepared as a "Tentative Method" and is made generally available. After a year or more of study and criticism by those interested, the tentative method, if found acceptable, is revised and presented in concise form to the Society for publication in Phytopathology as a "Recommended Method." Tentative methods not found acceptable are held for further study and modification. It is expected that the published Recommended Methods will be revised from time to time, if necessary.

The first Recommended Methods are appearing in this issue of PHYTOPATHOLOGY. Information concerning methods under current consideration either as Tentative or Recommended may be seen in the reports of the Committee to the Annual Meeting of the Society as published in PHYTOPATHOLOGY. Mimeographed copies of the Tentative Methods and reprints of the Recommended Methods may be obtained from the Committee Chairman (for address see Annual Report).

The Committee welcomes suggestions regarding new methods suitable for standardization.

DOSE, DOSAGE: Quantity of toxicant applied per unit of plant, fungus, lumber, soil, or special surface (6, 10). DOSE RATIO: Ratio between successively increasing doses (10). LD50: The median lethal dose, that is, the dose that kills 50 per cent of a large group of individuals (13). In case of spore germination tests—the dose that inhibits the germination of 50 per cent of the potentially viable spores (15). It is to be noted that a low LD50 VALUE indicates a high fungicidal value.

DEPOSIT: Quantity of dry fungicide[2] deposited on a unit area of plant, plant part or other surface, at any given application. The dried fungicide deposit remaining from the dose minus the run off. RUN OFF: Quantity of fungicide which runs off a unit area of plant, plant part or other surface during and immediately succeeding application. RESIDUE: Amount of dry fungicide remaining on a unit area of plant, plant part or other surface at any given time. DEPOSIT BUILDER: Material added to a fungicide to increase deposit rather than to increase tenacity.

ADHERENCE: Property of a fungicide to adhere or stick to a given surface. TENACITY: Property of a fungicide deposit or residue to resist removal by weathering (2). Tenacity is dependent on four major factors: adherence and insolubility of fungicide, nature of surface, and nature of weathering. TENACITY INDEX: The measure of tenacity. The ratio of quantity of fungicide residue per unit area of surface at the end of a given amount of weathering to that present at the beginning. STICKER: Material added to a fungicide to increase tenacity rather than to increase deposit.

SPREAD: Uniformity and completeness with which the fungicide deposit covers a continuous surface, such as a single leaf or seed. COVERAGE: Distribution of a fungicide deposit over a discontinuous area, such as leaves of a tree, or seeds. SPREADER: A material added to a fungicide to improve the spread of the liquid over a given area.

DILUENT: The component of a spray or dust that serves to reduce the concentration of active component and may aid in mechanical application, but does not directly influence toxicity. FILLER: A diluent in powdered form. CARRIER: A material serving as a vehicle for a fungicide.

SUPPLEMENT, *Syn.* ADJUVANT, AUXILIARY: A material added to a fungicide to improve some physical or chemical property (3, 11, 12). That is, a sticker, spreader, or deposit builder (see above), wetting agent, deflocculating agent, emulsifier, activator, or safener, but not the diluent. WETTING AGENT: A material that reduces the contact angle of a liquid on a given surface. Compare Spreader (3). Use wetting agent in preference to WETTER. DEFLOCCULATING AGENT: A material added to a fungicide suspension to delay sedimentation (12), in case of PROTECTIVE COLLOIDS, presumably by adsorption on the particles, and with DISPERSING AGENTS, presumably by dispersing aggregations of amorphous particles (11). EMULSIFIER: A material added to increase or stabilize the dispersion of one liquid within another. ACTIVATOR: A material added to a fungicide to increase either directly or indirectly its toxicity. SAFENER: A material added to a fungicide to eliminate or reduce phytotoxic effect. Used in preferenc to CORRECTIVE.

AVERAGE PARTICLE SIZE: (a) The arithmetic mean diameter. (b) the diameter of particle of average surface; (c) the diameter of particle of average volume; (d) the diameter of particle of same specific surface as the material. It should be stated which of these four possible diameters (5) is actually measured.

FLOWABILITY: The property of flowing possessed by liquids, colloids, or dusts.

## REFERENCES


1. American Phytopathological Society. Report of the Committee on Technical Words. Phytopath. 30: 361–368. 1940.
2. FAJANS, E., and H. MARTIN. The incorporation of direct with protective insecticides and fungicides. II. The effects of spray supplements on the retention and tenacity of protective deposits. Jour. Pom. and Hort. Sci. 15: 1–24. 1937.
3. FREAR, DONALD E. H. Chemistry of insecticides and fungicides. D. Van Nostrand Co., Inc., (New York). 300 pp. 1942.
4. GLASSTONE, SAMUEL. Textbook of physical chemistry. D. Van Nostrand Co., Inc., (New York). 1289 pp. 1940.
5. GREEN, HENRY. The effect of non-uniformity and particle shape on "average particle size." Jour. Franklin Inst. 204: 713–729. 1927.
6. HORSFALL, J. G., J. W. HEUBERGER, E. G. SHARVELLE, and J. M. HAMILTON. A design for laboratory assay of fungicides. Phytopath. 30: 545–563. 1940.
7. ————, R. W. MARSH, and H. MARTIN. Studies upon the copper fungicides. IV. The fungicidal value of the copper oxides. Ann. Appl. Biol. 24: 867–882. 1937.
8. KEITT, G. W. Some problems and principles of orchard disease control. Jour. Econ. Ent. 29: 43–52. 1936.
9. MCCALLAN, S. E. A., and R. H. WELLMAN. Fungicidal versus fungistatic. Contrib. Boyce Thompson Inst. 12: 451–463. 1942.

10. ————, ————, and FRANK WILCOXON. An analysis of factors causing variation in spore germination tests of fungicides. III. Slope of toxicity curves, replicate tests, and fungi. Contrib. Boyce Thompson Inst. 12: 49–78. 1941.
11. MARTIN, HERBERT. The scientific principles of plant protection. Edward Arnold & Co. (London). 3rd ed. 385 pp. 1940.
12. SHEPARD, HAROLD H. The chemistry and toxicology of insecticides. Burgess Publishing Co. (Minneapolis, Minn.). 383 pp. 1940.
13. TREVAN, J. W. The error of determination of toxicity. Proc. Roy. Soc. (London) B. 101: 483–514. 1927.
14. WHETZEL, H. H., and S. E. A. MCCALLAN. Studies on fungicides. I. Concepts and terminology. New York (Cornell) Agr. Exp. Stat. Mem. 128: 3–7. 1930.
15. WILCOXON, FRANK, and S. E. A. MCCALLAN. Theoretical principles underlying laboratory toxicity tests of fungicides. Contrib. Boyce Thompson Inst. 10: 329–338. 1939.

# THE SLIDE-GERMINATION METHOD OF EVALUATING PROTECTANT FUNGICIDES[1]

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, COMMITTEE ON STANDARDIZATION OF FUNGICIDAL TESTS

The method of evaluating protectant fungicides in the laboratory by means of spore germination tests on sprayed slides has undergone considerable development since its first application over 30 years ago (28, 30). It is now the most extensively used of all methods for testing the fungistatic (15) or spore inhibiting ability of protective fungicides (14, 27). During recent years in particular the method has been subjected to a critical analysis (10, 11, 16, 20, 21, 25, 31) which has resulted in marked improvement; so that at present it may be considered a precise method fulfilling the demands of modern bioassay. The errors associated with spore germination tests of fungicides arise from two sources: biological and mechanical. A rigid control and standardization of the methods of producing and germinating the spores have greatly lessened the biological errors (11, 16, 20, 31), while recent developments in precision apparatus for applying sprays and dusts have materially reduced the mechanical variation (9, 10, 11, 16, 21).

The various specifications as outlined below are considered to embody the best current techniques. However, in order to meet the requirements and facilities of different laboratories it has seemed desirable for the present to offer, in certain instances, various alternate procedures. All of the factors specified have been found to be of importance and must be considered when using this method. Reference is given to the original papers for a detailed discussion of the various procedures, which should be consulted before attempting to employ this method.

## GENERAL DESCRIPTION

The fungicide is applied to chemically clean glass slides by means of a precision technique such as a settling tower or horizontal sprayer. The former is suitable for sprays or dusts, the latter for sprays only. The deposition is regulated to give a series of dosages varying in geometric progression. The slides are allowed to dry and then placed in moist chambers. Fungus spores obtained under controlled conditions as regards species, strains, medium, age, temperature, concentration and stimulant if desired, are suspended in distilled water and pipetted onto the sprayed or dusted slides. The moist chambers are sealed with water and held at a temperature suitable for germination. In the case of water-soluble chemicals, the spores are added directly to the solution, and an aliquot then pipetted onto untreated slides. After a specified time the slides are placed under the low power of the microscope and the spores examined for germination. The percentage spores inhibited from germinating based on a specified count are plotted on logarithmic probability paper and the LD50 or LD95 obtained for comparing the relative merits of the different fungicides. Tenacity or rain resistance may be determined by subjecting the dried sprayed, or dusted slides to a laboratory "weathering" and comparing their toxicity to unweathered slides.

## GLASSWARE

*Cleaning.* All glassware, slides, racks, moist chambers, test tubes, pipettes, beakers, etc., must be chemically clean. The use of potassium dichromate-sulphuric acid mixture is

[1] Copies may be obtained from the Committee Chairman, Boyce Thompson Institute, Yonkers, New York.

satisfactory, but the glassware must be thoroughly rinsed at least five times in tap water followed by once in distilled water (13, 20). Chromium has marked fungistatic properties (15) and all must be removed. The glassware should be stored under dust-proof conditions.

*Glass Slides.* For ordinary purposes, 3 × 1 inch slides are used. Do not handle surface with fingers.

*Special Slide Surfaces.* Fungicides of low surface tension may require special type slides in order to control the area of fungicide in contact with the spores. Ordinary slides may be coated with cellulose nitrate (5, 8, 12, 13, 23, 24), or circles may be etched on the slides (26), cover glasses mounted on the slides (27), or cavity slides employed. Extreme care should be taken when attempting to delimit the spread of the drops that the materials used do not possess toxic or stimulant properties (13). Most of these special slides cannot be subjected to "rain" tests. However, if dilute cellulose nitrate, *i.e.*, 0.25 per cent in butyl acetate, is used to coat the slides they may be so exposed.[2]

*Slide Racks.* Racks to support the glass slides in the moist chambers may be made of solid glass rods bent into a U. Relatively unreactive sheet metal racks, such as aluminum, are also useful and more durable than glass.

*Moist Chambers.* Medium (200 mm. outside diam.), small (150 mm.), or large (250 mm.) moist chambers may be used. The chambers are inverted for use and a filter paper disc may be placed on the bottom to provide a background and to hold the slide rack in position. After placing the spore suspension drops on the slides the chambers are sealed with water. Do not place different fungicides, or different concentrations of the same volatile fungicide in the same chamber. Because of this, space is wasted in large chambers. A chamber set up with rack, slides, and drops of spores is illustrated in a paper by McAllan (13, p. 11).

## APPLICATION OF FUNGICIDE

*Sprays and Dusts.* Sprays and dusts must be applied by means of a precision apparatus, either a settling tower (9, 21) or a horizontal sprayer (5, 10, 11, 21). The former is suitable for both sprays and dusts, the latter for sprays only. Settling towers are more precise and in general more elaborate than horizontal sprayers, while horizontal sprayers are more rapid for one or two test fungi and permit greater variability in dose ratio. Other methods of applying sprays such as the use of a micro pipette (26) or a solid glass rod with ground top (27) have been employed. Likewise, test tube dusters have been used commonly for applying dust fungicides (13). However, until these techniques can be demonstrated to be equal or better in precision than the settling tower or horizontal sprayer, they cannot be advocated for a standard method.

*Settling Towers.* Settling towers function on the principle of filling the tower uniformly with the spray or dust and then allowing it to settle on the glass slides. Fungicide suspensions contained in a beaker and constantly stirred are sprayed up into the tower through an atomizer nozzle located near the base of the tower. When the tower is filled with a fine suspension, spraying is stopped and a preliminary settling period allowed for the heavy drops to fall. The slides are then introduced at the bottom. After a given period of exposure, the slides are withdrawn and the tower evacuated by forced draft. The entire operation is then repeated and a series of fungicide deposits obtained on the slides according to the number of times they were exposed. It is necessary to standardize on a number of factors such as dimensions of tower, spraying pressure, time of spraying, time of preliminary settling, time of exposure, and positional effect, if any, of slides. The de Vilbiss No. 15 atomizer is considered standard for the settling tower and horizontal sprayer. A satisfactory settling tower of known precision is discussed in detail in a paper by McAllan (21). Every individual settling tower or horizontal sprayer when constructed must be calibrated; in addition, a special calibration may be necessary for fungicides of unusual physical nature.

Settling towers for dusts are modified in that a given quantity of dust is placed in a "gun" and shot upward into the tower by sudden release of air pressure. An apparatus that has given satisfaction in different laboratories is described (9) in detail.

*Horizontal Sprayers.* A horizontal sprayer is a stationary apparatus in which the fungicide is sprayed horizontally through an atomizer nozzle onto a facing glass slide held a set distance away. The distance is within the range 20 to 30 inches. The duration of spraying is precisely controlled by a cut-off valve or stop cock, and the deposition of fungicide is determined by the time of spraying. The apparatus is suitably housed to eliminate stray air currents, back pressure, and to maintain a high humidity. As in the settling tower, the fungicide is held in a beaker and continuously stirred, and the nozzle is a de Vilbiss No. 15. Sprayers of tested precision are discussed and illustrated (10, 11, 21).

*Soluble Materials.* Soluble materials not to be tested for tenacity may be applied by means of the test tube dilution technique (16). A series of dilutions is prepared in

[2] Unpublished data furnished by J. G. Horsfall.

test tubes and to a known quantity, usually 2 cc., of the chemical, 0.5 cc. of spore suspension is added and two individual drops of the resulting suspension pipetted onto the glass slides. If necessary, orange juice or other stimulant is added along with the spores. The concentration of chemical should be expressed as per cent or parts per million by weight of (a) toxic ion or radical, or (b) total fungicide. This technique is more rapid than the horizontal sprayer or settling tower, though less precise, and may be used for preliminary orientation tests of readily suspendable materials. Care should be taken to agitate the suspension of fungicide and spores before withdrawing the pipette sample.

*Dose Ratio.* Dosages, *i.e.*, settling tower exposure, spraying time, or concentration of soluble materials should be varied in geometric progression. Standard ratios of $\sqrt{2}$, *i.e.*, 1.414, 2, 4, $\sqrt{10}$, *i.e.*, 3.16 or 10 are recommended (15, 16). Sufficient dosages should be run so that at least three finite germination responses will be obtained (16). Steep toxicity curves will require small dose ratios, and flat curves, large ratios.

*Deposition.* Express deposition of sprays and dusts as milligrams or micrograms of (a) toxic ion or radical, or (b) total solids, deposited per square centimeter of surface (8, 11, 16).

## STANDARDIZATION OF FUNGUS

*Species.* The following species have been used extensively and give satisfactory results (16): *Alternaria solani* (Ell. & Mart.) Jones & Grout, Delaware strain (16, footnote 5), *Glomerella cingulata* (St.) sp. & von S., *Macrosporium sarcinaeforme* (Cav.), and *Sclerotinia fructicola* (Wint.) Rehm. In addition, *Penicillium expansum* Link, and *Rhizopus nigricans* Ehr. + strain recently have been found suitable (15). All of these species may be used to test heavy metal and organic fungicides; only *Sclerotinia fructicola* is suitable for sulphur fungicides. In general, the use of at least two different species is recommended (16). Under the conditions specified below, the conidia of all six species should give a high percentage of germination in the controls, at least 95 per cent, except *Glomerella cingulata*, for which 90 per cent or higher should be obtained. Other species may be used provided they meet the requirements of reproducibility of results, ease of counting, and production of spores set forth (16, p. 70–72).

*Culture Media.* All six species may be cultured on test tube slants of potato dextrose agar (8, 15, 16, 20, 27) though oatmeal agar slants may be preferred for *Macrosporium sarcinaeforme* (3, 11).

*Age of spores.* The viability of most fungus spores changes markedly with age (4, 13). Spores from all species should be obtained from 7-day-old cultures (16, 20) except for the slower growing *Macrosporium sarcinaeforme*, where 14- to 21-day-old cultures are recommended (8, 11).

*Density of Spore Suspension.* It has been shown that the percentage germination response varies with the dose per spore and that there is a direct relation between the log. LD50 and the density of spore suspension (11, 16). Hence it is necessary to control the number of spores in suspension. The spore suspension concentration can be determined in a Fuchs-Rosenthal blood counting cell (20), and the final concentration should be adjusted to 50,000 spores per cc. This suspension will give about 35 spores per low power field (15 × ocular, 16 mm. obj.) (16).

*Application to Slides.* The spore suspension is applied to the slides by means of a 1 cc. or 2 cc. pipette, care being taken to maintain the spores in suspension. Two pairs of drops may be placed on each slide, the four drops being in a staggered position (13). In the case of settling tower or horizontal sprayer, the first pair of spore suspension drops are of one fungus species and the second pair of another species, while with the test tube dilution technique the two pairs of drops may constitute two different doses of fungicide. The drop should be approximately 0.05 cc. in volume (11, 16). On plain glass the drops should spread to a diameter of about 10 mm. (16), and on cellulose nitrate to a diameter of about 7.5 mm. (11). Difficulty will be encountered with materials of low surface tension and special surfaces will be required as stated above. In all cases, the area of the drops should be maintained reasonably constant within a test. Extreme departures from the above diameters should be stated.

*Temperature.* The six fungi specified may be cultured and will produce a maximum of spores at temperatures from 20° to 25° C. (11, 16). As is well known, spores vary in their temperature requirements for germination (4), and it has been shown (3, 22, 31) that their resistance to a fungicide is greatest when the temperature is optimum. However, it has been found that within the range 15° to 27° C. either *Alternaria solani* or *Sclerotinia fructicola* which have respectively high and low optimum temperatures will rate compounds in the same order (31). Accordingly, the temperature range 20° to 25° C. is specified for spore production and germination. Where it is of interest to obtain greater information regarding temperatures the spores should be tested at two or more temperatures that vary by at least 10° C.

*Stimulants.* It has been demonstrated that much of the day-to-day variation or replicate test error may be due to the presence of variable amounts of water-soluble

nutrient derived from the cultures when procuring the spores (20, 26, 27). In such cases the spores should be obtained by a vacuum technique or they should be washed and centrifuged to rid them of the contaminating stimulant (20, 26). Washed spores of some species, particularly *Sclerotinia fructicola*, will not germinate readily in distilled water (20, 27), hence it is necessary to add a known quantity of stimulant. Ultra-filtered orange juice used at a strength of 0.1 per cent has proved very satisfactory for most species. A concentration of 0.316 is preferred for *Penicillium expansum* and *Rhizopus nigricans* (15). A frozen supply of the orange juice may be kept on hand (20). Extracts from commercial dried potato dextrose agar (27) and coenzyme R (6) have also been used. The amount and nature of stimulant employed should be stated. Stimulants have been shown to increase the resistance of the spores to chemicals (3, 15), hence will affect the comparisons of different species.

## EXAMINATION FOR GERMINATION

*Time.* The spore may be examined for germination after 20 to 24 hours (18, 31). However, it has been found that a given fungus may rate fungicides in a different order at different periods of time, though usually no further change may be expected after 50 hours (31). Hence, when it is of interest to obtain greater information the spores should be examined at 6 hours and at 24 hours.

*Magnification.* A magnification of 100× is recommended for counting spores of *Macrosporium sarcinaeforme*, and 150× for *Alternaria solani*, *Glomerella cingulata*, and *Sclerotinia fructicola* (16). A cover slip is not required under ordinary circumstances.

*Number of Spores.* One hundred potentially viable spores should be counted per deposit or concentration of each compound, and for each species, provided it has been established that the error for replicate counts in the given laboratory is approximately that of the binomial distribution (17, 20). Otherwise, a replicate count of a second 100 spores is desirable. The count of 100 spores will consist of approximately 50 spores from each of the two spore suspension drops. Potentially viable spores are those that would germinate under the normal conditions of the control. A simple correction for potentially viable spores in the case of high germinating controls is as follows: count as many extra spores as the control is short of 100, and deduct this number from the ungerminated results. For example, if control gives 97 per cent germination, count a total of 103 germinated and ungerminated spores and subtract 3 from the ungerminated results. A more precise correction for lower controls is: corrected per cent germination = observed per cent germination × 100/control per cent germination (31). The counting of germinated and ungerminated spores is greatly expedited by means of a double hand tally counter, which may be operated by one hand and will record both classes.

*Length of Germ Tube.* The spore is arbitrarily defined as germinated if the length of the tube exceeds half the minor diameter of the spore. An approximation of the average germ tube length in microns for each count of 100 spores may be recorded. However, since germ tube length and percentage of germination is usually highly correlated, little is gained by measuring germ tubes (14).

*Clearing the Microscopic Field.* In the case of heavy deposits of fungicides, it is frequently difficult to see the spores. Copper fungicides may be readily cleared by the addition of a drop of concentrated acid. Organic solvents are sometimes successful with organic materials. A solution of 50 per cent chloral hydrate and 0.02 per cent safranine has been found fairly successful for staining spores of *Sclerotinia fructicola* and rendering them more visible in suspensions of sulphur fungicides.[3]

*Recording Results.* In order to conform to standard procedures used in toxicity measurements of insecticides, drugs, etc., the percentage of spores inhibited from germinating should be recorded (14, 33).

## COMPARISONS

*Dosage-response or Toxicity Curve.* In most toxicological data it has been found that the distribution of individual lethal doses tends to be symmetrical when plotted against the logarithm of the dose (29, 33). Because of this symmetry, if the toxicity curve is plotted on logarithmic-probability paper a straight line will result. Thus the per cent spores inhibited from germinating should be plotted against the dose or concentration of the fungicide on the logarithmic-probability paper[4] and the best straight line drawn through the points giving greatest weight to those nearest the center (33). However, in some cases, the points will have a definite trend away from a straight line, either toward a curve concave upwards, convex upwards, or sigmoid (16), or of a more irregular shape (3). These departures from the normal straight line should be substantiated by replication. In such cases "broken" straight lines must be plotted. With the data plotted as straight lines it is possible to compare fungicides on the basis of dose for equivalent inhibition of germination or control (3, 16, 33).

[3] Unpublished data furnished by S. E. A. McCallan.

[4] Codex Book Co., Inc., Norwood, Massachusetts.

*Unit of Comparison.* The common unit of comparison is the LD50 or dose inhibiting the germination of 50 per cent of the spores (11, 16, 19, 29, 33). The LD50 can be most precisely determined for a given spore count (29), and is best adapted for comparing straight line or concave type toxicity curves and for materials having the same slope (16). The LD95 is more practical for comparing fungicides having curves of the convex or sigmoid or irregular type and dissimilar slope (3, 16). When the LD95 is used it should be so stated.

*Standard Fungicide.* "Standard Laboratory Bordeaux Mixture" may be employed as a basis for general comparisons and for determining "Bordeaux Coefficients" (2).

*Replications.* Because of day-to-day variations in the resistance level of the spores, tests should be replicated on different days using different lots of spores, rather than at the same time (11, 16, 17, 20). It is preferable to test with several different fungi twice rather than one fungus a number of times (16).

*Error Term.* In making statistical comparisons of the LD50 or LD95 of a different fungicide, the compound × replicate test interaction ordinarily will be used for the error term (16). For a further discussion of statistical treatment see (16, p. 74).

## DETERMINATION OF TENACITY

It is desirable to determine the tenacity of fungicides after they have been subjected to laboratory weathering tests. The tenacity evaluated is dependent not only on the physical chemical properties of the fungicide, namely adherence and insolubility, but also on the two basic variables, nature of surface sprayed and method of weathering.

*Nature of Surface.* Glass (14, 19, 26) and cellulose nitrate surfaces (7, 23) commonly have been used. Information is not available as to which more closely resembles leaf surfaces in general or any leaf in particular.

*Method of Determination.* There are two basic methods of weathering to determine tenacity: (a) "artificial rain" and (b) immersion. The "artificial rain" may be applied by allowing water under pressure to flow upward through a rose-type nozzle and thence downward by gravity onto slides oscillated or rotated beneath (19), or it may be applied directly through a horizontal sprayer (24). The immersion method may consist simply of suspending the slides in a beaker of water for a short (32) or long (23) period of time, or of shaking the slides during or after immersion or both (7, 26). It would appear that "artificial rain" more closely simulates natural weathering, but immersion is simpler and more rapid. Limited tests indicate that these methods differed but little in their ability to remove the fungicide deposit (7). The procedure employed should be stated in detail.

*Tenacity Index.* The tenacity index is the ratio of active fungicide remaining after rain tests to that originally present (1). It may be determined by customary chemical analyses or by spore germination tests (7). In the latter case spores are germinated on unweathered slides and on a comparable set of weathered slides. The tenacity index is then derived (7) as follows: $\frac{\text{LD50 for unweathered slides}}{\text{LD50 for weathered slides}}$. The tenacity index may also be expressed on the basis of the LD95 and should be so stated.

## REFERENCES


1. American Phytopathological Society, Committee on Standardization of Fungicidal Tests. Definitions of fungicide terms. Phytopath. 33: 624–626. 1943.
2. American Phytopathological Society, Committee on Standardization of Fungicidal Tests. Standard laboratory Bordeaux mixture. Phytopath. 33: 633–634. 1943.
3. DIMOND, ALBERT E., JAMES G. HORSFALL, J. W. HEUBERGER, and E. M. STODDARD. Role of the dosage-response curve in the evaluation of fungicides. Connecticut Agr. Exp. Stat. Bull. 451. 635–667. 1941.
4. DORAN, WM. L. Effect of external and internal factors on the germination of fungous spores. Bull. Torr. Bot. Club 49: 313–340. 1922. (*Also in* New Hampshire Agr. Exp. Stat. Sci. Contrib. 19: 313–340. 1922.)
5. EVANS, A. C., and H. MARTIN. The incorporation of direct with protective insecticides and fungicides. I. The laboratory evaluation of water-soluble wetting agents as constituents of combined washes. Jour. Pom. and Hort. Sci. 13: 261–292. 1935.
6. GOLDSWORTHY, M. C., and E. L. GREEN. Effect of low concentrations of copper on germination and growth of conidia of *Sclerotinia fructicola* and *Glomerella cingulata*. Jour. Agr. Res. [U.S.] 56: 489–505. 1938.
7. HEUBERGER, JOHN W. A laboratory biological assay of tenacity of fungicides. Phytopath. 30: 840–847. 1940.
8. ———, and JAMES G. HORSFALL. Relation of particle size and color to fungicidal and protective value of cuprous oxide. Phytopath. 29: 303–321. 1939.

9. ————, and N. TURNER. A laboratory apparatus for studying settling rate and fractionation of dusts. Phytopath. 32: 166–171. 1942.
10. HORSFALL, JAMES G. Biological assay of protective fungicides. Chronica Bot. 6: 292–294. 1941.
11. ————, J. W. HEUBERGER, E. G. SHARVELLE, and J. M. HAMILTON. A design for laboratory assay of fungicides. Phytopath. 30: 545–563. 1940.
12. ————, R. W. MARSH, and H. MARTIN. Studies upon the copper fungicides. IV. The fungicidal value of the copper oxides. Ann. App. Biol. 24: 867–882. 1937.
13. MCCALLAN, S. E. A. Studies on fungicides. II. Testing protective fungicides in the laboratory. New York (Cornell) Agr. Exp. Stat. Mem. 128: 8–24. 1930.
14. ————. Methods of testing protective fungicides in the laboratory. Mimeo. report submitted to The American Phytopathological Society, Committee on Standardization of Fungicidal Tests, December, 1938.
15. ————, and R. H. WELLMAN. Fungicidal versus fungistatic. Contrib. Boyce Thompson Inst. 12: 451–463. 1942.
16. ————, R. H. WELLMAN, and FRANK WILCOXON. An analysis of factors causing variation in spore germination tests of fungicides. III. Slope of toxicity curves, replicate tests, and fungi. Contrib. Boyce Thompson Inst. 12: 49–78. 1941.
17. ————, and FRANK WILCOXON. The precision of spore germination tests. Contrib. Boyce Thompson Inst. 4: 233–243. 1932.
18. ————, and ————. The form of the toxicity surface for copper sulphate and for sulphur in relation to the conidia of *Sclerotinia americana*. Contrib. Boyce Thompson Inst. 5: 173–180. 1933.
19. ————, and ————. Laboratory comparisons of copper fungicides. Contrib. Boyce Thompson Inst. 9: 249–263. 1938.
20. ————, and ————. An analysis of factors causing variation in spore germination tests of fungicides. I. Methods of obtaining spores. Contrib. Boyce Thompson Inst. 11: 5–20. 1939.
21. ————, and ————. An analysis of factors causing variation in spore germination tests of fungicides. II. Methods of spraying. Contrib. Boyce Thompson Inst. 11: 309–324. 1940.
22. MCCLELLAN, W. D. Temperature as it affects spore germination in the presence of copper and sulphur. Phytopath. 32: 394–398. 1942.
23. MARSH, R. W. Notes on a technique for the laboratory evaluation of protective fungicides. Brit. Mycol. Soc. Trans. 20: 304–309. 1936.
24. ————. Some applications of laboratory biological tests to the evaluation of fungicides. Ann. App. Biol. 25: 583–604. 1938.
25. MARTIN, HUBERT. The significance of the bio-assay in studies of fungicidal action. Ann. App. Biol. 29: 326–328. 1942.
26. MONTGOMERY, H. B. S., and M. H. MOORE. A laboratory method for testing the toxicity of protective fungicides. Jour. Pom. and Hort. Sci. 15: 253–266. 1938.
27. PETERSON, P. D. The spore-germination method of evaluating fungicides. Phytopath. 31: 1108–1116. 1941.
28. REDDICK, DONALD, and ERRETT WALLACE. On a laboratory method of determining the fungicidal value of a spray mixture or solution. Science 31: 798. 1910.
29. TREVAN, J. W. The error of determination of toxicity. Proc. Roy. Soc. (London) B. 101: 483–514. 1927.
30. WALLACE, ERRETT, F. M. BLODGETT, and LEX R. HESLER. Studies of the fungicidal value of lime-sulphur preparations. New York (Cornell) Agric. Exp. Stat. Bull. 290: 163–208. 1911.
31. WELLMAN, R. H., and S. E. A. MCCALLAN. An analysis of factors causing variation in spore germination tests of fungicides. IV. Time and temperature. Contrib. Boyce Thompson Inst. 12: 431–449. 1942.
32. WILLIAMS, ROBERT C. Laboratory method for measuring relative adhesive qualities of fungicidal dusts. Indus. and Eng. Chem. Anal. Ed. 1: 81–82. 1929.
33. WILCOXON, FRANK, and S. E. A. MCCALLAN. Theoretical principles underlying laboratory toxicity tests of fungicides. Contrib. Boyce Thompson Inst. 10: 329–338. 1939.

# STANDARD LABORATORY BORDEAUX MIXTURE[1]

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, COMMITTEE ON STANDARDIZATION OF FUNGICIDAL TESTS

In the laboratory evaluation of fungicides it is necessary to employ a standard fungicide (a) as a check on the reproducibility of the technique, (b) as a basis of comparison for new compounds, and (c) under specified conditions, as a means of adjusting day-to-day variations due to changes in the resistance level of the spores (2, 4, 8). It is typical of bioassay in general, that each population will vary from day to day. Here each population of spores will vary in its resistance to fungicides. Controlled conditions have reduced but not eliminated this day-to-day variation (2, 4, 5, 7). Where a suitable standard is carried with each test, it is possible to rate each compound in terms of the standard and thus correct much of the day-to-day variation or replicate test error (2, 4, 8).

A standard fungicide should fulfill certain requirements. It should be well known, readily obtainable, easy to prepare, of known composition, stable and of medium toxicity (2). In addition, for a precise comparison, it should be of essentially similar chemical composition, and have a toxicity curve of similar slope to the "unknown" fungicides with which comparisons will be made (4). It is apparent that no single material can fulfill all of these requirements and function as a universal standard. It has been shown that day-to-day variation can be adjusted effectively only when standard and unknown are of similar chemical composition and slope (1, 4). Hence, a general standard may be selected to check on reproducibility of technique and as a basis of comparison for new materials, but it will have only limited application in correcting day-to-day variation (4).

Bordeaux mixture, the most important member of the most important group of fungicides, the copper compounds, is presented (2) as a suitable standard laboratory fungicide under the conditions above outlined.

## PREPARATION OF STANDARD LABORATORY BORDEAUX MIXTURE

The variability in Bordeaux Mixture is eliminated by standardizing the composition of the ingredients, the method of preparation and the temperature. Stock solutions instead of solids are used to insure rapid and uniform reactions. Temperature is held constant between 20° and 25° C. and reagent quality ingredients free of carbonates are employed (2).

*Stock Copper Sulphate.* A 3.928 per cent solution of reagent quality $CuSO_4 \cdot 5H_2O$ (1.0% Cu) is made up in distilled water.

*Stock Lime Water.* A saturated lime water solution is prepared by suspending an excess of reagent quality hydrated lime in boiled distilled water. Agitate thoroughly to obtain saturation. Let settle. Keep in siphon bottle protected from $CO_2$ with soda-lime tower.

*Standard Bordeaux.* The volume ratio of stock copper sulphate and lime water should be 1 to 12 (mol. ratio 1 Cu to 1.65 Ca). The stock copper sulphate should be added to the stock lime water while stirring. The quantities in cc. of stock copper sulphate to be added to stock lime water and diluted to obtain varying percentages of copper are as follows:

[1] Copies may be obtained from the Committee Chairman, Boyce Thompson Institute, Yonkers, New York.

| Final volume in cc. | | | | | | Per cent copper |
|---|---|---|---|---|---|---|
| 250 | | 500 | | 1000 | | |
| Copper | Lime | Copper | Lime | Copper | Lime | |
| 5 | 60 | 10 | 120 | 20 | 240 | 0.02 |
| ..... | ..... | 5 | 60 | 10 | 120 | 0.01 |
| ..... | ..... | ..... | ..... | 5 | 60 | 0.005 |

## DEPOSITION AND LD 50 VALUES FOR STANDARD BORDEAUX

In comparing the Bordeaux standard and "unknown" fungicides, the materials should be applied by means of a standard precision technique such as the settling tower (6) or horizontal sprayer (2). The approximate deposition of copper in microg. per sq. cm. to be expected from a Standard Bordeaux containing 0.005 per cent Cu for the two techniques is, respectively, 0.05 per one exposure (settling tower) and 0.03 per second of time (horizontal sprayer). The LD50 values (8) for spores of *Alternaria solani*, *Glomerella cingulata*, *Macrosporium sarcinaeforme*, and *Sclerotina fructicola* tested under the standard conditions (2, 3, 4, 5, 6, 7, 8) of the slide-germination technique should range from approximately 0.2 to 0.5 micrograms copper per sq. cm.

## DETERMINATION OF BORDEAUX COEFFICIENT

The toxicity of copper compounds with toxicity-curve slopes similar to Standard Bordeaux Mixture may be expressed as Bordeaux Coefficients (2) according to the formula:

$$\text{Bordeaux Coefficient} = \frac{\text{LD50 deposition of copper in Standard Bordeaux}}{\text{LD50 deposition of copper in Fungicide X}}.$$

The coefficients thus obtained will vary from test to test or day to day less than the actual LD50 values. For example in 10 tests of red cuprous oxide the coefficient of variation for the LD50 values was 45.4 but expressed as Bordeaux Coefficients it dropped to 23.9 (2). Hence the day-to-day variability was adjusted to one-half by the use of a suitable standard.

The toxicity of fungicide materials markedly unlike Bordeaux Mixture in composition and slope of curve may be expressed as Bordeaux coefficients on the basis of total solids in each, in order to make general comparisons with the Standard. However, in such cases as stated above, effective correction for day-to-day variation is not likely to result.

## REFERENCES


1. BLISS, C. I., and H. P. MARKS. The biological assay of insulin. II. The estimation of drug potency from a graded response. Quart. Jour. Pharm. and Pharmacol. 12: 182–205. 1939.
2. HORSFALL, J. G., J. W. HEUBERGER, E. G. SHARVELLE, and J. M. HAMILTON. A design for laboratory assay of fungicides. Phytopath. 30: 545–563. 1940.
3. ————, R. W. MARSH, and H. MARTIN. Studies upon the copper fungicides. IV. The fungicidal value of the copper oxides. Ann. Appl. Biol. 24: 867–882. 1937.
4. MCCALLAN, S. E. A., R. H. WELLMAN, and FRANK WILCOXON. An analysis of factors causing variation in spore germination tests of fungicides. III. Slope of toxicity curves, replicate tests, and fungi. Contrib. Boyce Thompson Inst. 12: 49–78. 1941.
5. ————, and FRANK WILCOXON. An analysis of factors causing variation in spore germination tests of fungicides. I. Methods of obtaining spores. Contrib. Boyce Thompson Inst. 11: 5–20. 1939.
6. ————, and ————. An analysis of factors causing variation in spore germination tests of fungicides. II. Methods of spraying. Contrib. Boyce Thompson Inst. 11: 309–324. 1940.
7. WELLMAN, R. H., and S. E. A. MCCALLAN. An analysis of factors causing variation in spore germination tests of fungicides. IV. Time and temperature. Contrib. Boyce Thompson Inst. 12: 431–449. 1942.
8. WILCOXON, FRANK, and S. E. A. MCCALLAN. Theoretical principles underlying laboratory toxicity tests of fungicides. Contrib. Boyce Thompson Inst. 10: 329–338. 1939.

## A POSSIBLE REPRINTING OF SACCARDO'S SYLLOGE FUNGORUM

The Alien Property Custodian has recently announced (Science 97: 303–4. 1943) that many technical books and sets of books of Axis origin are available for republication. The procedure to be followed in obtaining necessary licenses and other details is given and it is clear that every encouragement will be given to bring about prompt reproduction of books of this kind.

To mycologists and plant pathologists this announcement immediately suggests the possibility of the reproduction of Saccardo's classical *Sylloge Fungorum* in usable form. This compendium of mycological descriptions is a "sine qua non," and the comparatively few sets now in use in the Americas are showing the effect of much use. Additional copies have been practically non-existent heretofore, only occasional sets appearing on the market at rare intervals and at exorbitant prices. There are undoubtedly many institutions as well as individual mycologists and plant pathologists who will welcome an opportunity to purchase a set of Saccardo. Even those libraries now possessing the work will likely desire an additional set to relieve the wear and tear on the original.

It has been ascertained that a satisfactory reproduction of the 25 volumes can be produced. If 100 subscriptions are obtained by September 1, the complete set of 25 volumes can be obtained at $200.00 per set; if 300 subscriptions are obtained, the price will be $150.00 per set. These prices are based on an offset edition, with the type block photographically reduced ten per cent.

In order that the undersigned may obtain an idea of the number of prospective purchasers, interested mycologists are requested to send in tentative subscriptions and to interest their respective institutions in doing likewise.

JOHN A. STEVENSON,
Bureau of Plant Industry, Soils
and Agricultural Engineering,
Beltsville, Maryland.

## SUMMER MEETING OF THE UPPER MISSISSIPPI VALLEY PLANT PATHOLOGISTS

A summer meeting of plant pathologists of the North Central States has been scheduled for August 12 and 13 at Purdue University, Lafayette, Indiana. Headquarters for the meeting will be at the Purdue Memorial Union Building on the campus where room reservations may be made. The program will be organized about the solution of problems and the exchange of ideas pertinent to the maximum production of food and fiber in the war emergency. The war committee and the national officers of The American Phytopathological Society are scheduled to meet with the Upper Mississippi Valley group.

# EVIDENCE FOR THE EVOLUTION OF PHYTOPATHOGENIC VIRUSES FROM MITOCHONDRIA AND THEIR DERIVATIVES. I. CYTOLOGICAL AND GENETIC EVIDENCE[1]

M. W. Woods and H. G. DuBuy

(Accepted for publication January 11, 1943)

## CYTOLOGICAL AND GENETIC EVIDENCE

### INTRODUCTION

All plant viruses that have been chemically isolated contain d-ribose nucleoprotein (1). Some of these viruses at least are monomolecular in nature. At present, there is divided opinion as to the origin of viruses. Whereas most workers in the past have inclined to the view that viruses are derivatives of parasitic microorganisms, there has long been a school holding that these entities are autocatalytic proteins of ultimate host-cell origin, although the exact proteins have never been clearly defined. Thus, Woods (20) proposed the enzymic theory, which was further advanced with modifications by Freiberg (9), and Vinson (19). In 1933 Vinson demonstrated that the virus of tobacco mosaic is protein in nature. Stanley (18) later reported the isolation of this protein in "crystalline" form. Bawden *et al.* (2) were the first to demonstrate the nucleoprotein structure of viruses. In a previous paper (21) we demonstrated a relationship between the chromoprotein complex of the plastids of normal cells and tobacco-mosaic virus nucleoprotein. In the following two papers (*cf.* 7) we present proof that a ribonucleoprotein is a part of the normal chromoprotein complex. Furthermore, by presenting cytological and chemical evidence that characteristics of plastid-controlled variegations are intermediate between those of normal plants and virus-diseased plants, we connect virus proteins phylogenetically, over the variegation-inducing agents (abnormal plastids or chondriocontes), with proteins of the normal plastids or chondriocontes.

The similarities in symptoms of certain plastid-controlled variegations and certain infectious mosaic diseases are often striking. In both types of disease the symptoms are not limited to the chlorophyll alone, but as Woods (20) and Davis *et al.* (6) have shown, other physiological processes are involved. Woods believed both types of disease to be fundamentally alike; a contention borne out by the present work. Once plastids have mutated, such mutated plastids cause leaf variegation (5, 6, 12, 17). As Guilliermond (10) and others have pointed out, the plastids of higher plants can be considered as differentiations of the chondriome. The term chondriome includes all mitochondrial elements of the cell and their derivatives. Thus the

[1] Scientific Paper No. A42, Contribution No. 1869 of the Maryland Agricultural Experiment Station (Department of Botany).

TABLE 1.—*List of investigated variegated species and clones arranged in approximate order of their position in the spectrum (cf. Fig. 1)*

| Variegated species and clone | Symptom Type[a] | Location in spectrum[b] | Homochondric variegated cells | | | | Heterochondric cells | | Graft transmission | Origin of clone |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | V.-i. plastids | | | Approx. reduction in avg. cell size | Inhibiting action of v.-i. plastids or chondriocontes on normal plastids | Approximate reduction in cell size | | |
| | | | Approx. size ratio normal/V.-i. | Color in immature cell | Color in mature cell | | | | | |
| *Weigela* sp. | P, S | I A | 1.0/1.0 | Lt. green | Lt. green | 15% or less | Appar. absent | ..... | Not tested | Hort. var. |
| *Lonicera japonica* Thunb. clone #2 | P, S | I A | 1.0/1.0 | Lt. green | Lt. green to yellow | None | Do | ..... | Do | Field |
| *Ligustrum vulgare* L. | P, S | I B | 1.7/1.0 | Lt. green | Lt. green to yellow | None or very slight | Do | ..... | Do | Hort. var. |
| *Anthirrhinum majus* L. clone #1 | P, S, trace MS | I B | 1.7/1.0 | Lt. green | Lt. green to yellow | None or very slight | None to very slight | Ap. none | Do | Spontaneous |
| *Capsicum annuum* L. | S | I C | 2.0/1.0 | Lt. green | Lt. green to yellow | None or very slight | Not determined | ..... | Do | Spontaneous |
| *Xanthium* sp. (Tourn.) L. clone #1 | S, MS | II A | 1.3/1.0 | Lt. green | Yellow | Do | None to slight | None or very slight | Do | Field |
| *Lonicera japonica* Thunb. clone #1 | S, ext. MS | II B | 1.5/1.0 | Lt. green | Yellow | None | None to very slight | None | Negative | Field |
| *Trifolium pratensis* L. | S, MS | II C | 2.0/1.0 | Lt. green | Yellow to colorless | 17% or less | Pronounced | 15% or less | Not tested | Field |
| *Ambrosia trifida* L. | S, ext. MS | II C | 1.5/1.0 | Lt. green | Yellow | None or slight | Slight to pronounced | None or slight | Negative | Field |

TABLE 1—(*Continued*)

| Variegated species and clone | Symptom Type[a] | Location in spectrum[b] | Homochondric variegated cells | | | | Heterochondric cells | | Graft transmission | Origin of clone |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | V.-i. plastids | | | Approx. reduction in avg. cell size | Inhibiting action of v.-i. plastids or chondriocontes on normal plastids | Approximate reduction in cell size | | |
| | | | Approx. size ratio normal/V.-i. | Color in immature cell | Color in mature cell | | | | | |
| *Antirrhinum majus* L. clone #2 | P, S, MS | III A | 2.0/1.0 | Pale yellow | Colorless | 15% or less | Not determined | ..... | Negative | Spontaneous |
| *Rubus occidentalus* L. | S, ext. MS | III A | 3.0/1.0 | Pale yellow | Colorless | None or very slight | Not determined | ..... | Not tested | Spontaneous |
| *Xanthium* sp. (Tourn.) L. clone #2 | S, ext. MS | III A | 3.0/1.0 | Pale yellow | Colorless | 20% or less | Slight to pronounced | 20% or less | Not tested | Field |
| *Opuntia* sp. Mille | P, S, MS | III A | 1.2/1.0 | Pale yellow | Colorless | None or very slight | Not determined | ..... | Not tested | Horticulture |
| *Hedera helix* L. | Nearly P, S, MS | III A | 1.7/1.0 | Pale yellow to colorless | Colorless | 10% or less | Pronounced | 10% or less | Negative | Horticulture |
| *Peperomia* sp. Ruiz and Pav. | P, S, MS | III B | 3.5/1.0 | Pale yellow | Colorless | None or slight | Pronounced | None or slight | Not tested | Horticulture |
| *Cucumis melo* L. | S | IV A | Extremely small to chondriocontal | Colorless | Colorless | 25% or less | Not determined | ..... | Not tested | Spontaneous? |
| *Euonymus radicans* Sieb. clone #1 | P, S, MS | IV B | Chondriocontal | Colorless | Colorless | 30% or less | Very pronounced | 30% or less | Positive | Horticulture |
| *Lonicera japonica* clone #3 (= *Lonicera brachypoda reticulata* Nichols) | Vein-yellow | V | Subchondriocontal units. Apparently homochondric variegated cells cannot exist. | | | ..... | Very pronounced | None or very slight | Positive | Horticulture |
| *Nicotiana tabacum* L. | MS | VI | " | " | " | ..... | Slight to very pronounced | Slight to very pronounced | Positive | Mechanically infected plants |

[a] P = periclinal; S = sectorial; MS = Mosaic-sectorial.
[b] Capital letters indicate relative positions within the regions of the spectrum.

proplastids of the embryonic cell are considered as specialized mitochondria ("active chondriosomes" or "chondriocontes" *sensu* Guilliermond). Since chondriocontes have a definite heredity and are capable of mutation, they have fundamental properties in common with viruses. These properties of chondriocontes are probably under control of the ribose nucleoprotein component, which we have isolated from the normal plastid. The present paper furnishes cytological and other evidence for the origin of phytopathogenic viruses from the chondriome.

## MATERIALS AND METHODS

Variegated clones of the various species investigated were obtained from greenhouse stocks or collected in the field[2] (Table 1). Whenever possible, clones were established from both green and variegated shoots of the same plant species, and, where possible, also from the same plant. In many instances it was possible to compare normal and variegated tissues in the same leaf. Plants were grown in the greenhouse in sand culture and supplied with appropriate nutrient solutions. Material for microscopic study was aspirated in freshly prepared 10 per cent neutral Formalin in distilled water, and sectioned with a razor. This fixative preserved the nuclei and cytoplasmic structures in a manner closely resembling their appearance in living cells. Living cells, prepared in dilute nutrient solution, were compared with fixed material. An aqueous solution of iodine in potassium iodide was the most satisfactory stain for mitochondria and plastids. In some instances Janus green B was used as a vital stain for mitochondria. Grafts and bud transfers were made with techniques appropriate to the plant material involved.

## EXPERIMENTAL

### Symptoms of Variegation

Symptom patterns in variegated leaves were either periclinal, sectorial, or mosaic sectorial, (Fig. 1, Fig. 8, A). In the sectorial types varigated areas alternate or form a mosaic with normal tissues, whereas in the periclinal types the normal tissues occur as a central core surrounded by variegated cells or *vice versa*. In many plants the onset of variegation is characterized by sectorial variegation, which changes to a stabilized periclinal form maintained by vegetative propagation (Fig. 8, B). Massey (17) has provided an explanation for this conversion to the periclinal form on ontogenetic grounds. Certain variegated clones, however, remain mosaic-sectorially variegated (*e.g., Lonicera japonica* (Fig. 1, region II) and *Xanthium* clone 2) (Table 1). In these variegations the symptoms most closely resemble those occurring in such infectious diseases as *Abutilon* and tobacco mosaic. Some variegations such as those occurring in *Euonymus* may continue to develop all

[2] We acknowledge the aid rendered by Miss H. M. Christensen, Mr. C. E. Cox, Mr. R. Degen, Mr. J. Haney, Dr. J. B. S. Norton, Mr. W. L. Smith, Mr. R. Stewart, Mr. D. L. Stoddard, Dr. F. L. Wellman, and Mr. I. S. Zalph in collecting many of the clones investigated.

three major symptom types. As will be shown below the symptom pattern of variegation depends largely upon the behavior of the abnormal chondriocontе or plastid during leaf ontogeny.

## HISTOLOGY AND CYTOLOGY OF VARIEGATED TISSUES

### Histology

The symptom pattern of variegation can generally be explained on the basis of distribution of variegation-inducing chondriocontes[3] during division

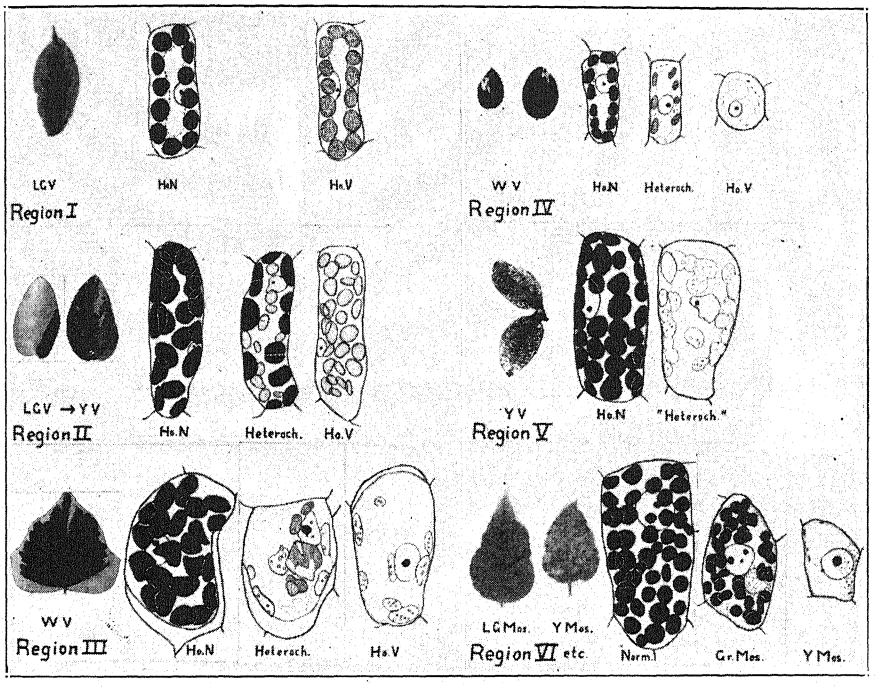



FIG. 1. Six types of variegations or viroses, representing 6 regions of a spectrum of variegation. Successive regions are based on progressively more drastic modifications in the variegation-inducing plastid (loss of pigments, loss of normal function, and reduction in size and chemical complexity). In each case photographs of leaf symptoms are accompanied by camera-lucida drawings (× 2000) of cells with normal plastids only (homochondric-normal = Ho.N), cells with both normal and variegation-inducing plastids or chondriocontes (heterochondric), and cells with variegation-inducing plastids or chondriocontes only (homochondric-variegated = Ho.V). In region VI a noninfected tobacco cell is shown together with a light green mosaic-infected and a yellow mosaic-infected cell. LGV = light green variegation; YV = yellow variegation; WV = white variegation. Illustrative species are as follows: region I, *Weigela* sp.; II, *Lonicera japonica* clone 1; III, *Hedera helix;* IV, *Euonymus radicans;* V, *Lonicera japonica* clone 3, and VI, *Nicotiana tabacum* var. Turkish, light-green and yellow tobacco mosaics (*Marmor tabaci* H.).

of pre-existing normal or variegated mother cells, and can be interpreted on a chimaeral basis. The composition of the mesophyll varied considerably in different parts of the same leaf or in different leaves of the same clone. Certain mosaic-sectorial patterns, however, could not be interpreted ade-

[3] Hereafter the term v.-i. will be substituted for the expression "variegation-inducing."

quately on a simple chimaeral basis. These were observed, for example, in *Lonicera japonica* clone 1 *Xanthium canadense* clone 2, and particularly in *Enonymus radicana* clone 1 (*cf.* Fig. 1).

*Normal Cells* (*Homochondric Normal Cells*). In normal cells (Fig. 1, Ho.N) the chloroplasts appeared as flattened spheres containing many minute pigment-bearing discs (grana) imbedded in a colorless stroma. The chloroplasts usually formed a single layer with their broad axes parallel to the cell wall, all available space being occupied. Starch grains could readily be delineated with IKI.

*Completely Variegated Cells* (*Homochondric Variegated Cells*). In completely variegated cells (Fig. 1, Ho.V) the variegation-inducing plastids were generally smaller than the chloroplasts of the normal cells, and often more numerous per cell and *light green in color*. The range of variation is summarized in table 1. In mild variegations (Fig. 1, region I of the "spectrum") the plastids of the mature cells remained green and were occasionally reduced in size. Distinct grana were sometime present, but starch grains were usually small or lacking. Cell size or shape were not markedly affected in most cases. In more extreme variegations (Fig. 1, regions II to III of the "spectrum") the pigment content of the v.-i. plastids was further reduced, as was their size. The cell size was often affected. The variegated cells contained more plastids than normal cells. For instance in *Lonicera japonica* clone 2, in which the cell size does not change the average number of normal plastids per unit cell area (32 cells measured) was $14.3 \pm .3$. In variegated cells (28 cells measured) the number of vi-i. plastids was $16.0 \pm .4$ per unit area. The fact that homochondric variegated cells contain more plastids than the corresponding normal cells indicates a higher division rate for the v.-i. plastids. The plastids of cells sometimes contained a small amount of chlorophyll (Fig. 1, region II), although in mature cells they were either *yellow or colorless*. In very old cells the v.-i. plastids were generally colorless and often highly vacuolate (Fig. 2). In the most extreme variegations the chondriocontes failed to develop beyond the mitochondrial stage and were always without pigment (Fig. 1, region IV). In these cases cell size and shape were generally markedly affected. In the types of variegation of regions II to IV of figure 1 changes in cell physiology were further evidenced by increased sensitivity of the tissues to adverse environmental conditions (mechanical and chemical injuries, desiccation, etc.), and development of vacuolar anthocyans. Abnormal vacuolation of the cytoplasm in old cells, and increased oxidase activity as indicated by browning of the cells of young leaves were observed. *In general the picture of cell pathology became more virose-like in proportion to the increasing abnormality of the v.-i.-inducing plastids or chondriocontes* (*cf.* 8). This was particularly marked in cells containing *both* variegation-inducing and normal plastids. These will be referred to as heterochondric cells.

*Partly Variegated or Heterochondric Cells.* Heterochondric cells have not been observed in some of the mildest variegations (Table 1, region I). In

some of the variegations of region I and in all of region II the v.-i. plastids did not cause marked pathological changes in the normal chloroplasts of the heterochondric cells. However, as the cell aged the normal plastids occasionally became lighter green. As in completely variegated cells, heterochondric cells with a high proportion of v.-i. plastids often showed evidence of physiological abnormality.

Heterochondric cells in more extreme variegations (Fig. 1, regions III to IV) are interesting because they most closely resembled cells infected with

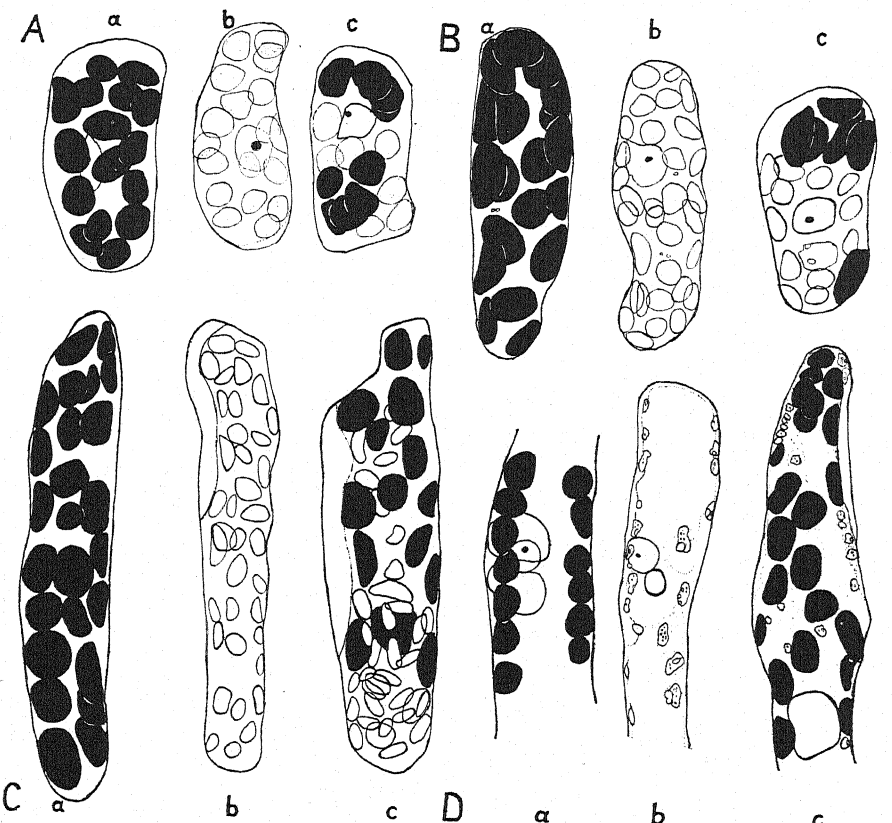



FIG. 2. A to D. Successive developmental stages of homochondric normal cells (Aa, Ba, Ca, Da), homochondric variegated cells (Ab, Bb, Cb, Db), and heterochondric cells (Ac, Bc, Cc, Dc) of *Lonicera japonica* clone 1. In this variegation the v.-i. plastids do not cause marked changes in the normal type plastids of heterochondric cells. Note progressive reduction in size of the v.-i. plastids with age of cell. Differences in cell size in this case are not significant.

"typical viruses." In *Xanthium* sp. clone 2, for example, the v.-i. plastids in the mature cell were colorless and rarely contained starch (Fig. 3, C). In heterochondric cells from mosaic-sectorial areas marked reduction in the size, the chlorophyll content, and the starch content of the normal chloroplasts was sometimes noted. Cell size also was often reduced. Adjacent cells, which contained only normal plastids, always appeared entirely

normal. The extent of derangement in the normal plastids of heterochondric cells was in proportion to the relative number of v.-i. plastids present in the same cells. It is significant, however, that in *Xanthium* sp. clone 2, and in certain other species, the occurrence of even a large proportion of v.-i. plastids in heterochondric cells did not always result in visible derangement of the normal chloroplasts. In this respect the behavior of the v.-i. plastids parallels the action of certain viruses in that the presence of the virus may or may not, depending on conditions of cell metabolism, cause visible changes in the chloroplasts of infected cells.

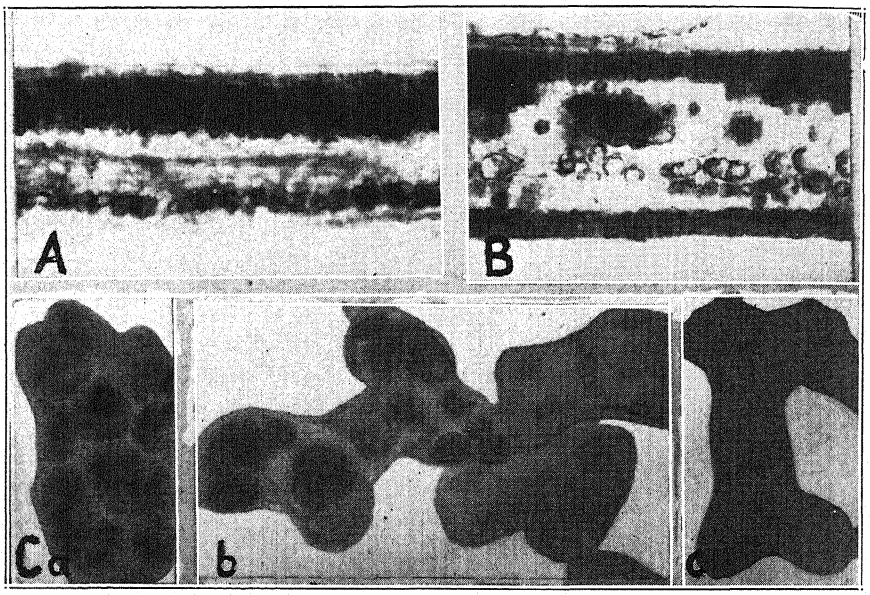



FIG. 3. A. *Lonicera japonica* clone 1. Sector of leaf with central mesophyll made up of homochondric-variegated cells. B. *Euonymus radicans*. Central mesophyll of homochondric-variegated cells. (Note similarity to A.) C. *Xanthium* sp. clone 2. a, homochondric normal cell; b, heterochondric and adjacent homochondric-variegated cells; c, homochondric variegated cell.

The variegation-inducing chondriocontes of the most extreme variegations (table 1, region IV), exert, like certain viruses (4, 8), a very strong inhibitory action on the normal chloroplasts occurring in the same cell. Thus, in white variegated clones of *Euonymus* (Figs. 4 and 5), the v.-i. chondriocontes, which never developed beyond the mitochondrial stage, completely inhibited the development of the normal plastids in the embryo cell. In such cases the maturation of the whole cell was markedly affected.

## EVIDENCE FOR INTERCELLULAR MIGRATION OF V.-I. CHONDRIOCONTES

### Transition Areas

In some of the rapidly growing immature leaves of *Euonymus radicans* clone 1, certain mosaic-sectorially variegated areas contain light-green cells

that form a transition zone between the normal-green and completely variegated tissues. These light-green transition zones contain cells with varying degrees of chloroplast inhibition, degeneration, or both (Fig. 1, Region IV, Heteroch.). There is likewise a gradation in degree of inhibition in cell growth (Table 2). The most severely affected cells occur nearest the com-

TABLE 2.—*Shape of palisade cells in transition zone between normal green and completely variegated (white) areas in a young leaf of Euonymus radicans. Note relationship between condition of the chloroplasts and shape of cell*

| Cell type | Dimensions of cells | | | |
|---|---|---|---|---|
| | Length | Width | Length/width | No. measured |
| Normal cells: normal chloroplasts ..... | 4.66 | 1.52 | 3.06 | 22 |
| Cells with degenerating chloroplasts | 4.49 | 1.68 | 2.67 | 16 |
| Cells without chloroplasts ..... | 3.24 | 2.32 | 1.39 | 18 |

pletely variegated tissues. Presumably, similar transition zones have been described by Hein (12) for *Scirpus, Diffenbachia, Dracaena, Croton, Abutilon* and *Plantago* sp. as gradual degeneration of the plastids on the margins of the discolored areas. These transition zones with heterochondric cells in *intermediate* stages of chloroplast breakdown and growth inhibition (Fig. 4) can best be interpreted by assuming that they were *invaded by v.-i. chondriocontes at some post-embryonic stage of ontogeny.* If these cells had been heterochondric from the beginning, cell size and shape would have been different from normal cells. No transition zone could be found in these cases (Fig. 5). The fact that this variegation, which shows transition zones,

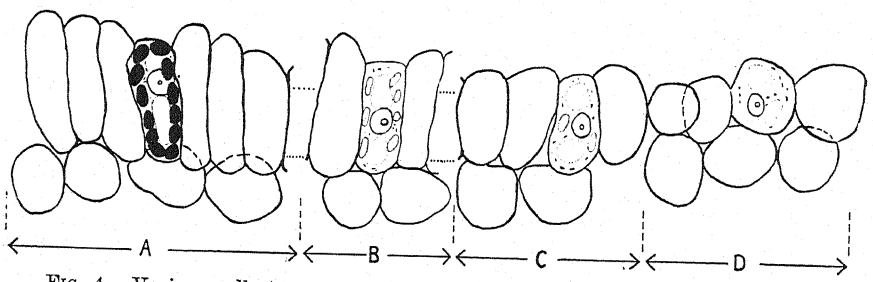



FIG. 4. Various cell stages of the transition zone of a mosaic-sectorial variegation of *Euonymus radicans:* A, normal cells; B, heterochondric cells; C, heterochondric cells with strongly degenerated "normal" plastids; D, totally variegated cells. (Semidiagrammatic camera-lucida drawing.)

has been graft-transmitted supports this view (Figs. 4 and 5). It should be emphasized, however, that intercellular migration of v.-i. chondriocontes in this plant seems to be very limited as most of the symptom pattern is clearly of the chimaeral type encountered in the other "non-transmissible" variegations (Fig. 3B and 5). Indications of intercellular migration of v.-i. chondriocontes in certain mosaic-sectorial variegations of the less severe type (Regions II and III, Fig. 1) were found, but the cytological evidence could be interpreted with less certainty. *Lonicera japonica* clone 1 (Figs. 3, A,

and 6) was the most favorable object for these studies, since both normal and v.-i. plastids could be distinguished readily in the heterochondric cells. In this case they exerted little or no degenerative action on the normal chloroplasts of mature cells. However, the inability to use changes in cell size as a "marker" for determination of the time of invasion was a disadvantage. In this clone the chief indications of limited intercellular migration were the occurrence of small isolated irregular patches or islands of normal cells surrounded by a diffuse transition zone of heterochondric cells in otherwise completely variegated areas. Furthermore, there existed a pronounced tendency for masses of both heterochondric and homochondric variegated cells to be delimited by the leaf veins. In the first case the highest frequency of v.-i. plastids occurred in the transition zones nearest the surrounding com-

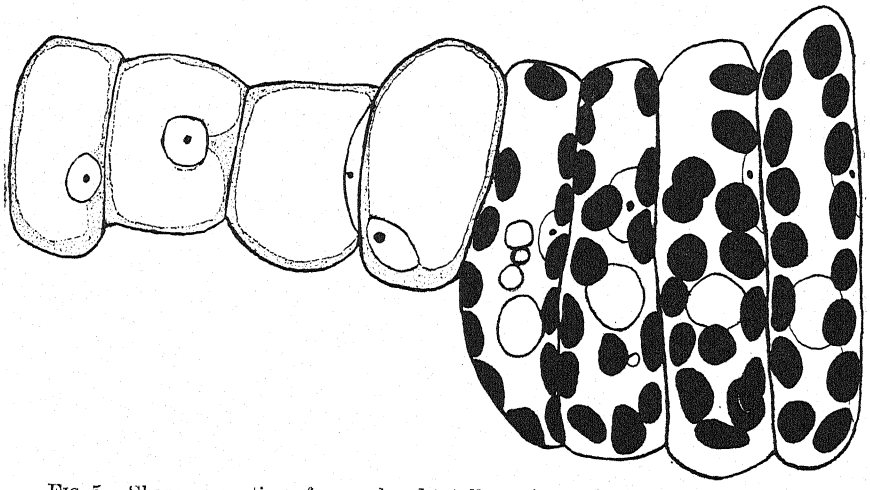


FIG. 5. Sharp separation of normal and totally variegated cells of a leaf of *Euonymus radicans*. Notice difference in cell size. (Semidiagrammatic camera-lucida drawing.)

pletely variegated tissues, indicating that progressive invasion into the normal cells during early ontogenetic stages had occurred. Since vascular tissues are differentiated very early it seems likely that migration of v.-i. chondriocontes may occur after the formation of the vascular strands, *but before maturation of the leaf,* since there is no extension of the variegated area in the mature leaf.

The establishment of v.-i. chondriocontes in normal cells seems to depend on the ability of the invading chondriocontes either to inhibit normal plastid development (as occurs in *Euonymus*) or on their ability to prevent further division of the normal chondriocontes or plastids, which are already present at the time of invasion. Whether daughter cells will be completely variegated may, then, depend on either segregation of normal and v.-i. chondriocontes or on early invasion followed by inhibition of the development of the normal plastids. The simplest explanation for those cases where new growth of certain variegated shoots becomes *progressively* more variegated and more mosaic-like is the occurrence of some invasion. These signs of invasion were

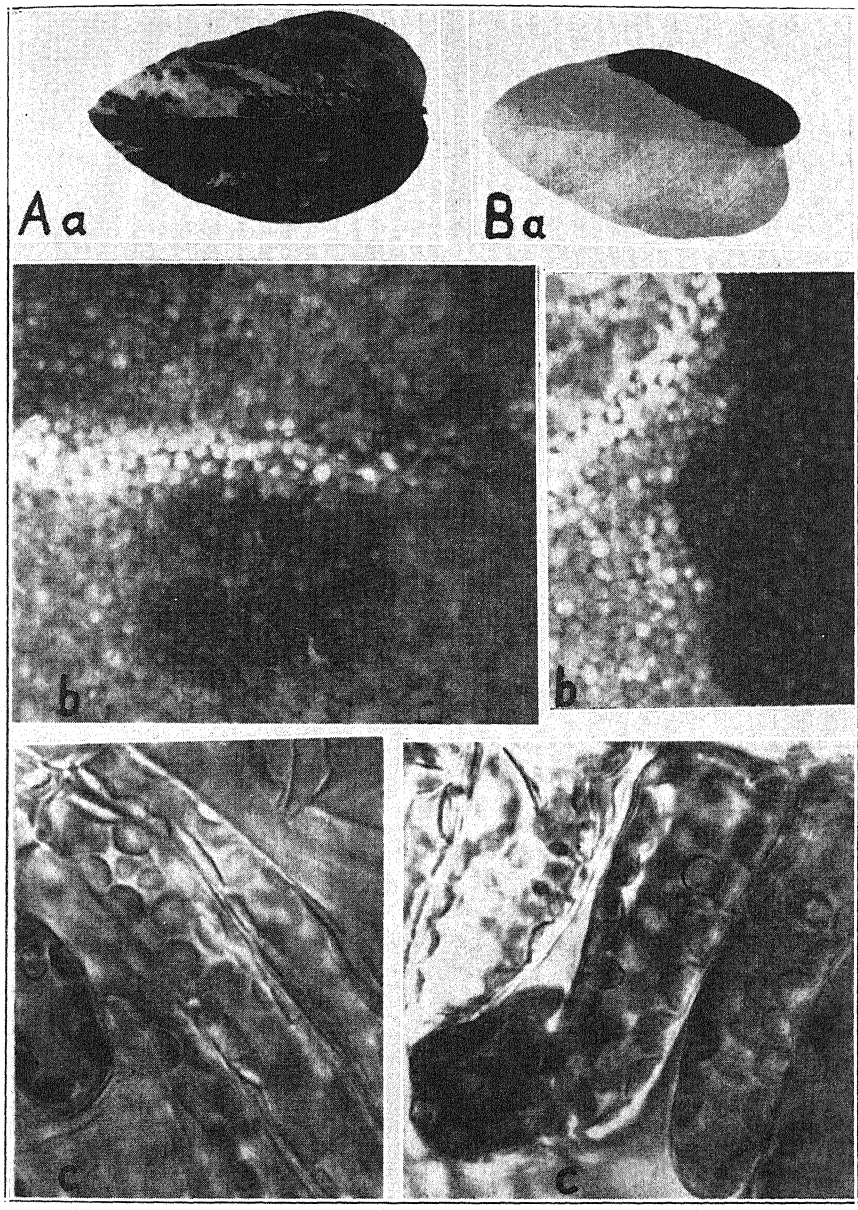



FIG. 6. *Lonicera japonica* clone 1: Aa, mosaic-sectorially variegated leaf; Ab, a mosaic sectorial area, × 400; Ac, section showing homochondric normal, heterochondric and homochondric variegated cells, × 2000. Note difference in size of normal and v.-i. plastids. Ba, sectorially variegated leaf; Bb, a sectorial area; Bc, adjacent homochondric normal and homochondric variegated cells. (Magnifications as under A.)

never observed in variegations of region I: these always showed relatively regular patterns of variegation. The signs of invasion become more pronounced the farther one proceeds in the spectrum. Thus in *Lonicera japonica* clone 2 (Region IA) the v.-i. plastids are slightly abnormal and the symptom pattern is still mainly regular. In *Lonicera japonica* clone 1 (Region IIB) as we have seen, the differences between the two type plastids are already more pronounced and mosaic patterns of variegation occur. The same is true for *Xanthium*. The most extreme case is that of *Lonicera japonica* in which the v.-i. agent is apparently invisible and transmission general.

In general, there seems to be a correlation between the degree of abnormality of the v.-i. chondriocontes or plastids (as indicated by size, color, or both) and their invasiveness as indicated by a mosaic pattern. *However, a small chondrioconte is not necessarily invasive* (see p. 645). Furthermore, the smaller the v.-i. chondrioconte, the stronger its effect upon the normal plastid. In other words, in order to invade, the v.-i. chondrioconte has to be small. Since small v.-i. chondriocontes have a strong effect upon the normal plastids, invasion, when it occurs, is always linked with the more pronounced changes of the normal plastids.

## GRAFT TRANSMISSION OF VARIEGATION

Graft transmission of chondrioconte-controlled variegation would afford direct proof of the ability of v.-i. chondriocontes to migrate intercellularly. So far in the present investigation graft-transmission studies have been limited to 5 species. The results indicate that, while graft-transmission of typical chondrioconte-controlled variegation can occur, such transmission is extremely difficult to accomplish. Thus in 10 attempts to transmit mosaic-sectorial variegation of *Lonicera japonica* clone 1, there was no evidence of transmission over 10 months after grafting. Three attempts to transmit the variegation of *Hedera helix* (Fig. 1), and 5 attempts to transmit a mosaic-sectorial variegation of *Ambrosia trifida* (Table 1) also failed although the variegated coins grew well for several months after grafting. The vein-yellowing variegation of *Lonicera japonica, L. brachypoda reticulata* Nichols of horticulture (14, 16) (Fig. 1), however, was graft-transmitted in more than 10 cases to several green varieties of *L. japonica*. The first symptoms usually developed on the stock plants 30 to 60 days after grafting. Movement of the variegation-inducing agent (as judged by symptom response) was through the vascular system. Secondary graft-transmission from graft-infected plants was readily accomplished. Plants infected 10 months earlier grew vigorously and are continuing to show symptoms on new growth.

Eight attempts to transmit the white mosaic-sectorial variegation of *Euonymus radicans* to *E. japonica* were made. Observations have been continued for over 200 days, but no evidence of successful transmission has occurred. However, of 6 attempts to transmit this variegation to green

clones of *E. radicans* 1 was positive. While the successful transmission involved only 1 plant, the evidence is entirely conclusive, as detailed records were made of the plant several months before and after grafting. The

TABLE 3.—*Graft-transmission of type IV variegation in Euonymus radicans. A single variegated shoot was grafted into the healthy stock plant*

| Days after grafting | Condition of graft-inoculated plant | | | |
|---|---|---|---|---|
| | Number branches on whole plant | Variegated shoots | Per cent of branches variegated | Severity of symptoms |
| 0 | ap. 40 | 0 | 0 | All healthy |
| 77 | 56 | 8 | 14 | Very slight |
| 137 | 91 | 12 | 13 | Slight to pronounced |
| 274 | 121 | 21 | 17 | Slight to pronounced |

results are summarized in table 3. The ingrafted diseased cion was about 3 cm. long and bore several heavily variegated leaves. Good union was secured between a nodal region of the cion and a sub-terminal node of a rapidly growing shoot on the healthy stock plant. The first symptoms were observed on the latter 77 days later in 8 separate branches of about the same age. Seven of the infected shoots bore one or two leaves each with small flecks of variegated tissue (Fig. 7, A, B). The apical meristems were

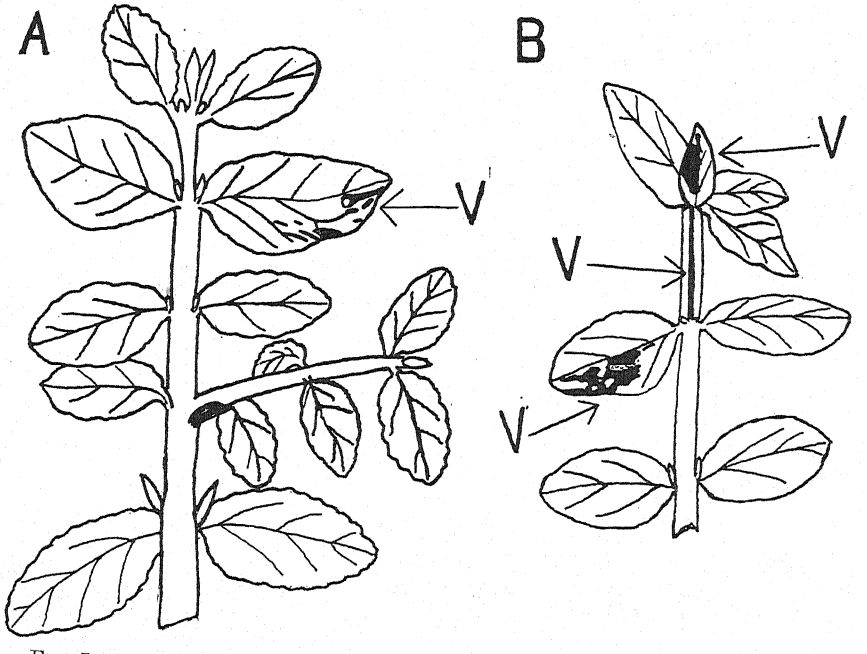



FIG. 7. Two shoots of *Euonymus radicans*, showing veriegated areas 77 days after transplanting a white mosaic-sectorially variegated shoot of the same species: A, leaf variegation only; B, leaf and apical meristem variegation.

not involved, except in the eighth shoot (Fig. 7, B). That the symptoms in each of these 8 branches were primary was evidenced by the complete absence of symptoms anywhere else on the plant at this time. The infected stem tip on the eighth branch was separated from the original ingrafted variegated cion by 35 nodes, or a distance of approximately 69 centimeters. The v.-i. agent presumably moved this distance through the vascular channels and then entered the parenchymatous cells of the leaf primordia or stem tip. It gradually continued to spread to other parts of the plant entering the apical meristems of several branches. The tendency for the v.-i. chondriocontes to remain localized is demonstrated by the subsequent behavior of apparently symptom-free cuttings, which were removed from the infected plant. Thus, 4 variegated cuttings removed 95 to 170 days after grafting produced only variegated plants in which 17 of a total of 29 branches were variegated, some heavily. Five green cuttings removed 92 to 152 days after grafting produced 3 variegated plants with 7 out of a total of 17 branches variegated (only traces of variegation in some), and 2 non-variegated plants with a total of 17 branches. On the original infected plant some very rapidly growing shoots have apparently grown away from the variegation. Similar localization of the virus of Abutilon mosaic and slow-moving strains of tobacco mosaic have been reported (15). In all of the variegated branches of the infected plants the symptoms are mosaic-sectorial or sectorial. Thus distribution of v.-i. chondriocontes in leaf parenchyma is chiefly by cell division, even though they may move long distances through the vascular tissues.

### SEXUAL INHERITANCES OF VARIEGATION

Studies of sexual inheritance of v.-i. chondriocontes were limited chiefly to *Antirrhinum majus*. While the detailed results will be published later by Miss J. L. Showacre, certain data are presented here. The light-green variegated clone, and the white clone 2 (Table 1) were chiefly investigated. In both types the inheritance of the v.-i. chondriocontes proved to be matroclinous. Flowers produced on periclinally variegated shoots (Fig. 8) gave completely variegated progeny, which soon died. Flowers of sectorially-variegated shoots (Fig. 8, A), however, produced normal green, completely variegated, and sectorially variegated seedlings (Fig. 8, C). Many of the sectorially variegated seedlings possessed enough green tissue to complete their development. In other tests 22 seedlings obtained from a periclinally variegated *Lycopersicum esculentum* were completely variegated and died. In other words, it seems that continued sexual propagation of variegation depends upon maintenance of the mosaic sectorial pattern.

### ORIGIN AND FREQUENCY OF VARIEGATION

Spontaneous origin of a recognized virus in a previously virus-free plant has never been proved. This is in harmony with a theory of their gradual evolutionary differentiation from chondrioconte nucleoproteins. The spon-

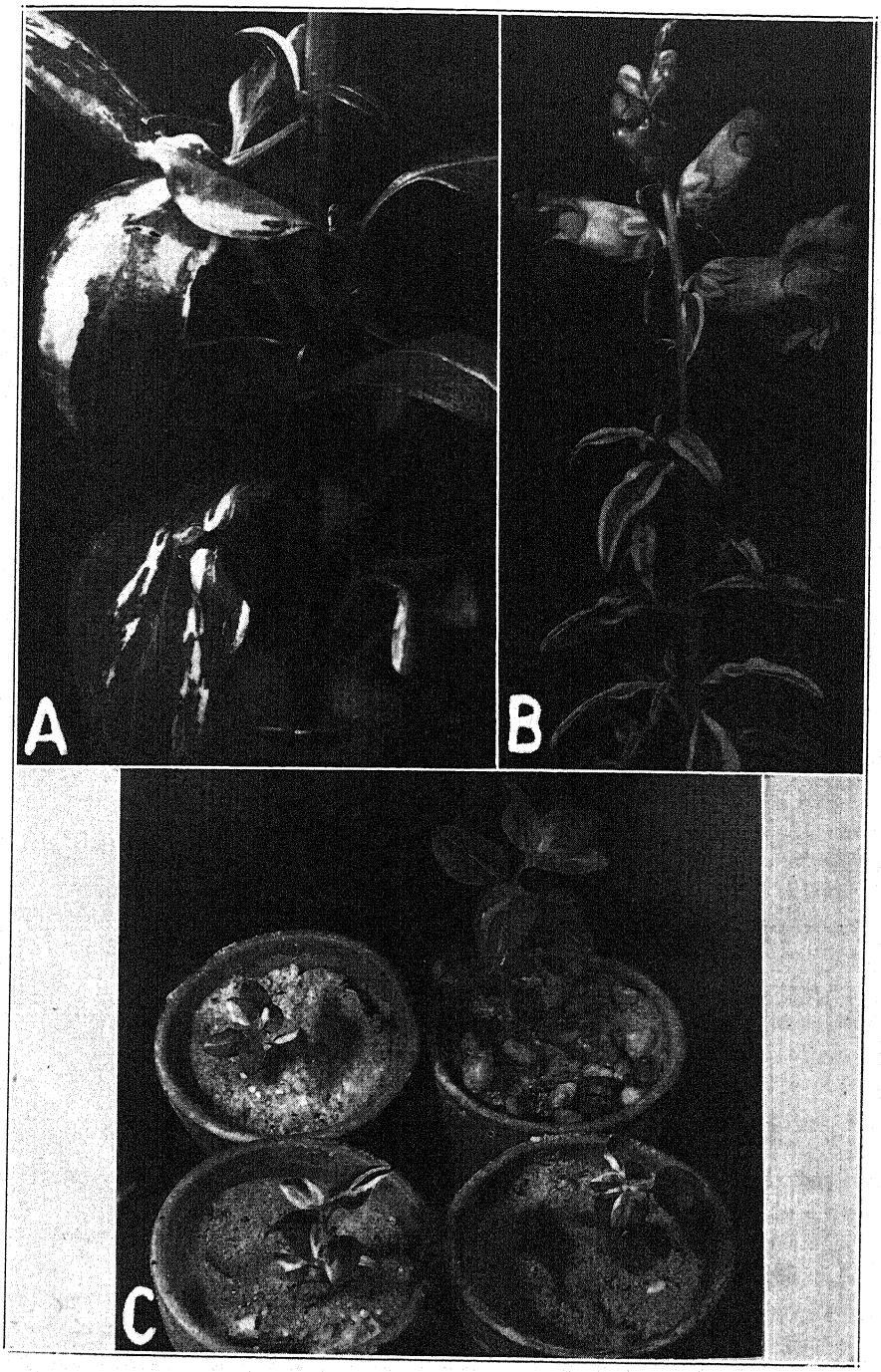



FIG. 8. A. Sectorially-variegated shoot, which produced sectorially-variegated seedlings (C). B. Periclinally variegated shoot which produced nonviable completely variegated progeny. (Courtesy of Miss J. Showacre.)

taneous development of chondrioconte-controlled variegations, on the other hand, has been observed in the present investigation. Thus the two variegated clones of Antirrhinum (Table 1) appeared in a progeny of approximately 1,000 seedlings from nonvariegated parents (we are indebted to Mr. F. B. Winkler for these clones). The variegated haploid clone of *Capsicum* (Table 1) arose as a single branch on the mother plant, which was over 6 months old. This haploid has been described in detail by Christensen and Bamford (3) from whom the variegated clone was obtained.

Field surveys of large populations of several species indicate that the frequency of variegation in nature is low (one in several thousand or million individuals), but more than enough to support the present theory. Approximate counts in selected regions of Maryland, for example, have shown that in *Ambrosia trifida* plastid mutation occurs relatively often (one in several thousand), whereas in *Xanthium* the frequency in most locations is much lower. One exceptional colony of *Xanthium* however, was found in which approximately 100 variegated individuals were observed in an area covering about one-half acre of ground. All of the variegated individuals but one were of the same white form. The one exceptional plant possessed light-green variegated plastids. Circumstantial evidence indicates that the variegated colony was produced by sexual reproduction. This is borne out by the occurrence of sectorially variegated seedlings in progeny obtained from 2 of these plants. So far only 2 mosaic-sectorial variegations have been found in *Lonicera japonica* (Table 1), although many thousands of plants have been examined. The vein-yellowing variegation in this species (Fig. 1) has been collected from 3 widely separated localities in Maryland, but each seems to represent an escape from an original clone that presumably traces back to a form long circulated in commerce (16). Also, over 20,000 black raspberry plants were examined in detail and only 2 cases of plastid-controlled variegation were found. In one locality over 3,000 plants of *Plantago lanceolata* were examined without finding a trace of variegation, although 2 sectorially variegated clones were found elsewhere. Information is rapidly accumulating indicating that almost any higher plant species may develop plastid-controlled variegation.

## DISCUSSION

That certain variegations involving the chloroplast are to a certain extent under plastid control has often been indicated (5, 6, 12, 17). So far as the writers are aware, however, previous evidence for intercellular migration of plastids or proplastids is considered inadequate. Our study indicates that when such migration does occur it is while the plastids are in a chondriosomal condition. Intercellular passage of these chondriosomes or their essential constituents could probably take place through plasmodesmata. The concurrence of all conditions requisite for intercellular passage of chondriosomes or their essential constituents would be realized only at a critical stage in the ontogeny of the cell. This stage probably occurs before

maturity of the tissues. That the chief means of distribution of chondriosomes in the leaf tissues, however, is through cell division is obvious from the chimaeral structure of variegated leaves. The actual demonstration of intercellular passage of chondriocontes or their essential constituents is probably not possible with existing techniques, but strong evidence that such intercellular movement of chondriocontes really does occur has been obtained in the present study. The facts constituting this evidence cannot be explained by existing theories of chondrioconte segregation. They consist of the 3 groups of observations in favor of intercellular migration, which are presented here: (1) the manner in which heterochondric cells are distributed in the symptom pattern of certain types of variegations, (2) the varying degrees of inhibition of cell development of heterochondric cells in the transition zones, which sometimes occur between normal and variegated areas (Fig. 4), (3) the transmissibility by grafting. This evidence for limited invasion allows a further comparison of chondrioconte-controlled variegations and viroses.

The most important obstacle to the establishment of a connection between variegations and viroses was the difference in migration of the agent, which causes the abnormalities. A detailed survey of the field of plant viroses, however, reveals that they can be arranged in a series in respect to a progressively more pronounced invasiveness (13). Those that are the least invasive (*e.g.,* Euonymus- and Abutilon-mosaic viruses) are also those that produce symptoms more closely resembling certain chondrioconte-controlled variegations. Thus, again, this comparison constitutes further evidence for the evolution of viruses from variegation-inducing chondriocontes.

Since chondriocontes have definite powers of mutation and heredity, they are subject to natural selection. Furthermore, since the v.-i. chondriocontes can multiply in previously normal cells, and may retard or prevent the development of normal chloroplasts in those cells, it is obvious that the abnormal chondriosomal types are able to compete with the normal types for substrate. A similar competition between tobacco mosaic virus and normal plastids has already been reported (21). This constitutes a further parallelism of variegations and viroses.

A review of the data already published and of new data obtained in the present study indicates strongly that mutations within the chondrioconte, coupled with natural selection of more stable, actively multiplying, and invasive types may have resulted in the occurrence of such slowly invasive and probably unstable viruses as those causing Euonymus mosaic, Abutilon mosaic, etc., and through more extreme modification in viruses like the tobacco-mosaic virus proteins, which are very stable and highly invasive. These former viruses may still possess the complex coacervate lipoid-protein structure of chondriosomes. *Thus certain viruses may be considered as derivatives of the chondriome, which have become modified through evolution, rather than as derivatives of parasitic micro-organisms.* This agrees with Dufrenoy's observations that a virus that causes localized necrotic

spots but fails to become systemic is a virus that fails to induce systemic liberation of the protein fraction from the lipoprotein stroma of the plastids (Phytopath. Zeitsch. **5**: 290–301, Fig. 7), whereas a virus that becomes systemic causes the protein in the plastids to become remobilized into amino acids, such as tyrosin and leucine (*l.c.*, Fig. 8; also P. Z. **2**, p. 384, Fig. II). Chondrioconte-controlled variegations, therefore, are not necessarily virus diseases, although they may be potential starting points for the evolution of viruses, particularly in perennial plants. For instance, graft-transmissible variegations, such as that present in *Euonymus*, might be considered as proto-viroses.

Further evidence of evolution would consist in the demonstration that the chondriosomal or plastid protein complex of higher plants contains a ribose nucleoprotein of the same character as the known virus-ribo-nucleoprotein. This proof will be presented in another paper (7).

While the possible occurrence of drastic mutations in the chondrioconte nucleoprotein, leading directly to the formation of a "monomolecular" virus cannot be excluded, the failure to demonstrate the spontaneous origin of any such viruses indicates that a more gradual type of evolution has occurred.

## SUMMARY

Certain plastid-controlled (chondriosome-controlled) variegations can be arranged into a series or spectrum, depending on the extent to which the plastid structure and function is modified. Consecutive regions of this spectrum show an increasing similarity to certain virus diseases.

In general, plastid-controlled variegations are characterized by typical sectorial or periclinal chimaeral structure in the variegated leaves. Many mosaic sectorial variegations occur, however, that suggest that variegation-inducing chondriosomes may actually invade normal cells by intercellular passage at some critical stage of ontogeny.

The variegation-inducing chondriosomes often behave like viruses with respect to their physiological action on the cell.

A variegation of *Euonymus*, that appears to be of the chondriosomally controlled type, was transmitted by grafting to a previously nonvariegated stock plant.

Variegations usually are inherited matroclinously, as are viruses.

It is suggested that viruses have been derived through mutation and natural selection from constituents of the chondriosomes rather than from preexisting parasitic microorganisms.

The spontaneous origin of variegation has been observed in several cases. Furthermore, the frequency of such plastid mutations in nature is sufficient to furnish a basis for the present theory.

DEPARTMENT OF BOTANY,
MARYLAND AGRICULTURAL EXPERIMENT STATION,
COLLEGE PARK, MARYLAND.

LITERATURE CITED

1. BAWDEN, F. C. Plant viruses and virus diseases. 272 pp. Chronica Botanica Co. (Leiden). 1939.
2. ————, N. W. PIRIE, J. D. BERNAL, and I. FRANKUCHEN. Liquid crystalline substances from virus-infected plants. Nature 138: 1051–1052. 1936.
3. CHRISTENSEN, H. M., and R. BAMFORD. Jour. Hered. 1943 (in press).
4. COOK, T. The effect of mosaic virus on cell structure and chloroplasts. Jour. Dept. Agr. Puerto Rico. 15: 117–182. 1931.
5. DALE, E. E., and O. L. REES LEONARD. Plastid variegation and concurrent anthocyanin variegation in *Salpiglossis*. Genetics 24: 356–367. 1939.
6. DAVIS, E. F. Some chemical and physiological studies on the nature and transmission of "infectious chlorosis" in variegated plants. Ann. Mo. Bot. Gard. 16: 145–226. 1929.
7. DUBUY, H. G., and M. W. WOODS. Evidence for the evolution of phytopathogenic viruses from mitochondria and their derivatives. II. Chemical evidence. Phytopath. 1943 (in press).
8. DUFRENOY, J. Le spotted-wilt. Ann. Epiphyties et de Phytogénétique. 3: 187–223. 1937.
9. FREIBERG, G. W. Studies in the mosaic disease of plants. Ann. Mo. Bot. Gard. 4: 175–232. 1917.
10. GUILLIERMOND, A. The cytoplasm of the plant cell. 247 pp. Chron. Bot. Co. (Waltham, Mass.). 1941.
11. HANSTEIN, K. Die Plastidenvererbung bei *Oenothera Berteriana* und *O. odorata*. Zeitsch. Md. Abst. Vererb. 75: 661–681. 1938.
12. HEIN, I. Changes in plastids in variegated plants. Bull. Torr. Bot. Club. 53: 411–418. 1926.
13. HOLMES, F. O. Handbook of phytopathogenic viruses. 221 pp. Burgess Pub. Co. (Minneapolis). 1939.
14. Illustration Horticole. 9: t. 337 (c). 43 vol. (Gand). 1859–96.
15. KEUR, J. Y. Studies of the occurrence and transmission of virus diseases in the genus *Abutilon*. Bull. Torr. Bot. Club 61: 53–70. 1934.
16. La Belgique Horticole. 21: 59, t. 20 vol. (Gand). 1851–70.
17. MASSEY, K. The development of the leaves in certain periclinally variegated plants. Jour. Gen. 19: 357–372. 1928.
18. STANLEY, W. M. Isolation of a crystalline protein possessing the properties of tobacco mosaic virus. Science 81: 644–645. 1935.
19. VINSON, C. G. Further purification of the virus of tobacco mosaic. (Abstract) Phytopath. 23: 35. 1933.
20. WOODS, A. F. The destruction of chlorophyll by oxidizing enzymes. Centralb. f. Bakt. u. Par. Krankheit. II. 5: 745–754. 1899.
21. WOODS, M. W., and H. G. DUBUY. Synthesis of tobacco-mosaic virus protein in relation to leaf chromoprotein and cell metabolism. Phytopath. 31: 978–990. 1941.

# ROOT ROTS IN STORAGE OF DECIDUOUS NURSERY STOCK AND THEIR CONTROL[1]

GEORGE Y. YOUNG[2]

(Accepted for publication December 30, 1942)

In Federal and State nurseries in the United States large quantities of numerous species of trees, shrubs, and vines have been grown annually for use in reforestation and in soil conservation. For the most part these were transplanted to their permanent locations in the early spring as 1-year seedlings. To make such seedlings available for spring planting, usually they are dug in the preceding fall and stored over winter in "heel-in" trenches outdoors or indoors in packing sheds, root cellars, or caves.

In outdoor heel-in beds, as well as in indoor storage in nurseries in the Upper Mississippi Valley, large quantities of nursery stock have deteriorated every year by molding and rotting of the roots. This is distinct from the stem rot reported by Gilman and Sproat[3] and was a more common cause of losses. In the winter of 1937–38 at an Iowa nursery the entire stock of black locust (*Robinia pseudoacacia* L.) in overwinter storage was lost from such injuries. At a Minnesota nursery the annual losses of black locust seedlings have equaled 20 to 50 per cent or more. Other species grown in nurseries, suffering from root rot in storage, were especially Osage orange (*Maclura pomifera* (Raf.) Schneid.), Russian mulberry (*Morus alba* f. *tatarica* (L.) Ser.), black walnut (*Juglans nigra* L.), wild plum (*Prunus americana* Marsh.), bur oak (*Quercus macrocarpa* Michx.), and tulip tree (*Liriodendron tulipifera* L.).

In the fall of 1938 a study was undertaken in an Iowa nursery to determine the cause of these losses. Plants of the species mentioned above and, in addition, green ash (*Fraxinus pennsylvanica* var. *lanceolata* (Berkh.) Sarg.), Siberian pea tree (*Caragana arborescens* Lam.), gray dogwood (*Cornus racemosa* Lam.), climbing bittersweet (*Celastrus scandens* L.), Siberian elm (*Ulmus pumila* L.), Tatarian honeysuckle (*Lonicera tatarica* L.), riverbank grape (*Vitis riparia* Michx.), and prairie rose (*Rosa setigera* Michx.), were subjected to a wide variety of storage conditions outdoors in heel-in beds of soil, sand, shingletow, and sawdust, mulched and unmulched, and indoors in packing sheds, root cellars, caves, and refrigerators. Indoors the plants were ricked or heeled-in in shingletow, sphagnum, or sand. In ricking (shelving) the plants were stored by the root-to-root method and

[1] This work was a cooperative undertaking between the Division of Forest Pathology, Bureau of Plant Industry; the Department of Botany and Plant Pathology, Iowa State College; and the Nursery Division of the Soil Conservation Service. This paper is based on a more detailed report by George Y. Young and Albert F. Dodge, "Storage Losses of Black Locust and other Deciduous Nursery Stock." Mimeograph, 43 pp. 1941.

[2] Assistant Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture; formerly Assistant Forest Pathologist, Civilian Conservation Corps, Bureau of Plant Industry, United States Department of Agriculture.

[3] Gilman, J. C., and B. B. Sproat. A *Fusarium* following frost injury of *Robinia*. Iowa Academy of Science Proceedings 43: 101–106. 1936.

some by the root-out method, and the roots of some were protected and those of others were left bare. Where a protective medium for the roots was used, different levels of moisture content were used. Temperature and relative humidity of storage also were recorded. Similar series were replicated in a Minnesota nursery where losses usually have been severe and a Missouri nursery where losses seldom were serious.

### DESCRIPTION OF THE ROOT DECAY IN STORAGE

One of the first symptoms of the root deterioration referred to above is a certain stickiness, noted on handling the affected roots. Later, they appear to be wet; water-soaked areas of a darker hue than the healthy areas then become manifest. The latter usually appear first on or near the root extremities. The water-soaked areas may appear, however, on any part of the root from the soil collar down. These lesions enlarge and may coalesce to form extensive affected areas. Later, desiccation and shriveling occur, and almost invariably a gray-white to pink mycelium covers the affected areas. When a partly shriveled and molded root is cut longitudinally, the area of decay is evident by the discoloration of the cortex. At a later stage of decay, when most of the root is molded, the cortical tissues become flaccid, and the cortex may be easily opened with the fingers without cutting. At or just prior to this stage of decay, the cortex tissues of the root become "thready" and may be separated into strands. Still later the entire root becomes soft and mushy. Only in very few cases has this condition of the root extended beyond the collar into the stem.

### STORAGE STUDIES WITH BLACK LOCUST

One phase of the work undertaken at Iowa was a study of the relation of the time of lifting of stock to the incidence of decay in storage. Plants of black locust were lifted at intervals in late fall and stored, both indoors and outdoors, under various methods of storage. The results of this work are summarized in table 1.

Examination of the table will show that in the packing shed nearly all of the root rot occurred in plants lifted on Nov. 7 and Dec. 14 when tempera-

TABLE 1.—*Percentage*[a] *of root rot in black locust as related to date of digging, air temperature, and storage treatment. Iowa, 1939*

| Date stock lifted | Temperature (°F.) on day of digging | | | Ricked in packing shed | Heeled-in | | |
|---|---|---|---|---|---|---|---|
| | | | | | In soil | | In shingle-tow outdoors |
| | Min. | Max. | 7 p.m. | | In root cellar | Out-doors | |
| Oct. 28 | 36 | 77 | 57 | 0.3 | 3.3 | 0.1 | 88.0 |
| Nov. 4 | 40 | 44 | 42 | ..... | 9.0 | 0.0 | 57.0 |
| Nov. 7 | 26 | 30 | 28 | 13.3 | ..... | ..... | ..... |
| Nov. 12 | 42 | 64 | 44 | 0.0 | 2.9 | 0.0 | 73.0 |
| Dec. 6 | 25 | 45 | 39 | 0.8 | 20.0 | 72.0 | 79.0 |
| Dec. 14 | 20 | 33 | 20 | 27.3 | 100.0 | 100.0 | 100.0 |

[a] Based on examination of 200 plants in each case.

tures below freezing prevailed at the time the plants were being lifted, since the maxima for these two dates are 30° and 33° F., respectively. Also, all plants stored in shingletow heel-in beds, outdoors, regardless of the date of lifting, suffered heavily from root rot. In the soil heel-in beds outdoors in the case where 72 per cent of the plants rotted, large air pockets were found in the heel-in trench due to faulty heeling-in, which resulted in the roots of these plants being inadequately protected from mass movements of cold air within the trench. For plants lifted on Dec. 6 it is possible that at least part of the stock was lifted in temperatures below freezing.

For the greater part of the work undertaken in Iowa the plants were dug on Nov. 12, with the temperature considerably above freezing (Table 1). Results of examination of some 10,000 plants are given in table 2.

TABLE 2.—*Percentage of root rot in black locust as related to method of storage. Iowa, 1939*

| | | |
|---|---|---|
| Indoor storage, all methods of storage | less than | 0.5 |
| Outdoor storage: | | |
| Heeled-in in soil | less than | 0.1 |
| Heeled-in in coarse sand, mulched | | 8.3 |
| Heeled-in in coarse sand, nonmulched | | 27.0 |
| Heeled-in in shingletow, mulched and nonmulched | | 100.0 |
| Plants left in nursery rows for spring lifting | | 0.0 |

The data in tables 1 and 2 show: 1. All plants lifted in temperatures above freezing and stored in adequate storage suffered little or not at all from root rot; 2. All plants lifted in temperatures below freezing, that is, plants whose roots were exposed to temperatures below freezing, all suffered heavily from root rot regardless of the method of storage; and 3. All plants stored outdoors in porous media, such as shingletow or coarse sand, that is, media that permit mass movements of cold air within the heel-in trench, suffered heavily from root rot. It appeared, therefore, that root-rot damage depended on whether or not the roots of the plants were exposed to temperatures below freezing, either at the time of digging or in improper storage.

As already noted, the storage studies undertaken at the Minnesota and Missouri nurseries were substantially the same as those undertaken in Iowa. Table 3 summarizes the results of the work in Minnesota.

TABLE 3.—*Root rot in black locust as related to storage method. Minnesota, 1939*

| Materials | Indoor storage | | Outdoor storage | | | Plants left in nursery rows for spring lifting |
|---|---|---|---|---|---|---|
| Nursery stock | Ricked and heeled-in in cellar | Ricked and heeled-in in cave | Heeled-in in sawdust | Heeled-in in shingle | Heeled-in in soil | |
| Root rot percentage[a] | 35.0 | 35.0 | 100.0 | 100.0 | 35.0 | 0.0 |
| Plants examined | 10,500 | 1,200 | 600 | 600 | 600 | 1,000 |

[a] Percentage figures are average values for several tests.

The losses in outdoor storage in shingletow and sawdust were total. In all other forms of storage the amount of root rot proved curiously uniform,

varying from about 25 to 40 per cent, and averaging close to 35 per cent for all tests. This unusual uniformity was explained when it was found that at this nursery it required 12 days to lift the stock of black locust and on 5 of these days freezing or below freezing temperatures prevailed. Thus, approximately 42 per cent of the stock was exposed to freezing or below freezing temperature while it was being lifted. After lifting, the stock was transported to temporary storage pending grading and culling. This piling up of plants lifted on different days, and the grading and culling operations mixed the plants lifted under different temperature ranges. Unwittingly, plants from this mixture were selected the preceding fall for the storage tests. In such a mixture one would expect something less than 42 per cent of the roots to have been damaged by freezing and the recorded root decay of 35 per cent approached this figure in all forms of good storage where temperatures after storage seldom were below 40° F.

At the Missouri nursery appreciable amounts of root rot occurred only in shingletow and sand heel-in outdoor beds that were not mulched. The results obtained at the Minnesota and Missouri nurseries were consistent with the freezing relations deduced from the work in Iowa.

### STORAGE STUDIES WITH OTHER DECIDUOUS SPECIES

The results of storage studies in Iowa with nursery species other than black locust are summarized in table 4.

Freezing injury of the roots apparently is the primary cause of root decay in storage of these species, as well as of black locust. In storage in shingletow outdoors the roots of all species except Siberian pea tree suffered heavily from root rot. In sand storage outdoors, a larger percentage of root rot occurred in the nonmulched than in the mulched beds. In soil, which was the densest heel-in medium used, the percentage of root rot was the least. In all indoor storage there was virtually no root rot, except for mulberry. Since this species also suffered heavily in soil storage, it is probable that at least part of the stock of mulberry was lifted while temperatures were below freezing.

It seems evident also that plants of different species vary in their tolerance of, or resistance to, low temperatures. Siberian pea tree, for example, escaped injury in all forms of storage where other species suffered much. Green ash is another, even hardier, species. The case of 77 per cent loss in shingletow storage is misleading. The molded condition of the roots was associated with severe injury to the roots by rodents. Osage orange, black walnut, and Russian mulberry are species that were found to be more susceptible to temperatures below freezing.

### EXPERIMENTAL EXPOSURES OF SEEDLINGS TO SUB-FREEZING TEMPERATURES

The close relationship observed between exposure of the roots of plants to sub-freezing temperatures and the amount of root rot in storage called for a direct test of this factor. A preliminary test is described below.

TABLE 4.—*Percentage of root rot in nursery seedlings as related to storage method. Iowa, 1939*

| Species | Indoor storage | | Outdoor storage | | | | |
|---|---|---|---|---|---|---|---|
| | Number of plants in test | Percentage of plants rotted | Number of plants in test | Percentage of plants rotted | | | |
| | | | | Heeled-in in soil | Heeled-in in sand | | Heeled-in in shingletow |
| | | | | | Mulched | Not mulched | |
| Osage orange | 3,000 | 0.0 | 1,800 | 31[a] and 61 | 84.0 | 100.0 | 100.0 |
| Black walnut | 1,600 | 0.0 | 1,200 | 9.8 | 59.0 | 59.0 | 100.0 |
| Russian mulberry | 1,600 | 31.0 | 1,200 | 45.0 | 36.0 | 100.0 | 100.0 |
| Wild plum | 1,600 | 0.0 | 1,200 | 0.0 | 0.0 | 0.0 | 100.0 |
| Siberian pea tree | 3,000 | 0.0 | 1,800 | 0.0 | 0.0 | 0.0 | 0.0 |
| Green ash | 3,000 | 0.0 | 1,800 | 0.0 | 0.0 | 0.0 | 77.0 |
| Bur oak | 1,600 | 0.0 | 1,200 | 10.0 | 0.0 | 0.0 | 100.0 |
| Gray dogwood | 500 | 0.0 | 100 | ..... | 0.0 | 0.0 | ..... |
| Riverbank grape | 1,600 | 0.0 | 1,200 | 0.0 | 0.0 | 0.0 | 100.0 |
| Prairie rose | 600 | 0.0 | 100 | ..... | 0.0 | 0.0 | ..... |
| Climbing bittersweet | 400 | 1.3 | 100 | ..... | 0.0 | 0.0 | ..... |

[a] 31 per cent in the mulched and 61 per cent in the unmulched trenches. For the other species the difference was not appreciable.

On a February morning with the temperature at 17° F., 160 healthy black locust seedlings were taken from indoor storage and spread outdoors. At intervals of 20, 35, 60, 120, 165, 240, 300, and 360 minutes, 20 plants were returned to indoor storage. Each lot of 20 plants was divided into 2 loose bundles of 10 plants each. To 1 bundle 2 locust plants with rotted roots were added to serve as inoculum and a source of infection, while none was added to the other bundle. After exposure, all plants were stored indoors in moistened shingletow. Unexposed plants, with and without rotted roots, served as controls. The temperature at the beginning of the test and at the end of each interval of exposure noted above was 17, 17, 17, 18, 20, 23, 25, 25, 25 degrees F.

In this test all plants exposed to these temperatures, even for the short period of 20 minutes, suffered heavily from root rot in storage. The average loss for the 8 intervals of exposure was 92.5 per cent, and in no case was the loss less than 80 per cent. The plants to which rotted material was not added rotted just as completely as did those to which this material was added. All 20 plants not exposed to outdoor temperature remained healthy and entirely free from rot.

This preliminary work left little doubt that exposure of the roots to temperatures below freezing was the primary condition for the development of root rot in storage. There remained the problem of determining in more detail the temperature and duration of exposure that will effect permanent injury to the roots.

Freezing tests by exposing plants of various species to artificially regulated sub-freezing temperatures were undertaken during the storage season of 1939–40. The thermostatically regulated refrigerator of the Botany and Plant Pathology Department of Iowa State College was used for this purpose. The results of this work are summarized in figure 1.

Plants of the species noted in figure 1 were exposed to the ranges of temperature indicated by the arrows for periods of 5, 10, 20, 30, 45, 60, 120, 240, and 360 minutes. Plants of *Robinia,* for example, were exposed for periods of 5 to 360 minutes to the temperature ranges given, which are: 11–13° F., 15–16.5° F., and 24–25° F. The roots of all plants of *Robinia,* in all tests, exposed for 20 minutes or longer to any of the temperature ranges given, deteriorated in overwinter storage. Likewise, plants of the genera *Ulmus, Maclura,* and *Morus* required an exposure of only 20 minutes to a temperature range of 24–25° F. to effect permanent injury and predispose the plants to root decay in storage. Plants of *Juglans* were exposed only to the temperature range of 11–13° F. For this temperature the critical level of *Juglans* is 30 minutes, that is, plants exposed for 5, 10 and 20 minutes all fared well in overwinter storage, indicating that they were not permanently injured, while for exposures of 30 minutes or longer, the roots rotted in storage. Plants of *Quercus,* exposed only to the temperature range of 10–13° F., succumbed to root rot only after an exposure of at least 60 minutes. Plants of *Caragana,* not noted in the diagram, were exposed

to a temperature range of 12–17° F., but did not succumb to root rot until after an exposure of 120 minutes or longer. Plants of *Fraxinus* all fared well in storage, even after an exposure of 360 minutes or six hours to a temperature of 12–14° F.

With plants of *Prunus* the results were somewhat erratic. In 2 tests these plants succumbed to root rot after an exposure of only 20 minutes to temperatures of 15–16.5° and 21–23° F. In a third test the roots of the plants did not rot until after an exposure of 45 minutes or longer to a temperature range of 12–17.5° F. The type of root deterioration also differed

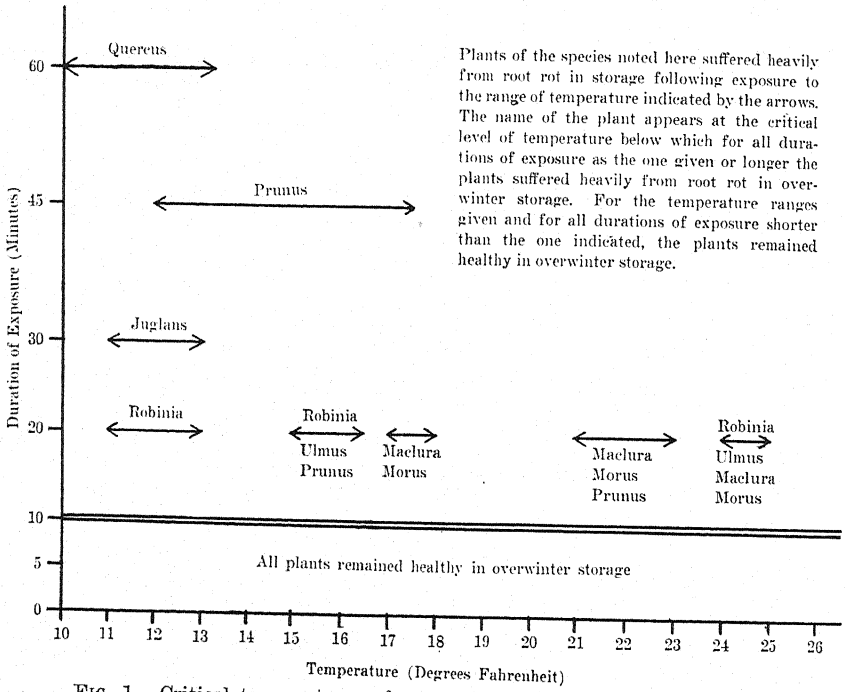



FIG. 1. Critical temperatures of exposure for various nursery species.

in various tests. The type described for *Robinia* occurred sometimes, while in other tests the roots simply would dry and subsequently die with blue or green molds covering most of the plants.

### THE ROLE OF FUNGI IN THE PRODUCTION OF ROOT ROT IN STORAGE

The tissues of rotted roots were almost invariably tinged with pink streaks, indicating the presence of *Fusarium* species. A Fusarium was always isolated from rotted roots. Other organisms isolated from rotted roots were species of *Alternaria, Mucor, Rhizopus, Penicillium,* a species of bacteria, and a fungus designated as isolate #6, which was not identified. Of these latter, *Alternaria* was the fungus most frequently isolated.

Each of the above-mentioned isolates was inoculated into 10 healthy seedlings of black locust through root incisions. The wound and inoculum were

wrapped with gauze and the plants were stored indoors in loose bundles of 10 plants protected with moist shingletow. Controls consisted of 10 additional healthy plants, similarly incised on the roots but without inoculum. After 60 days of storage the plants were examined and the following results were observed.

All 10 plants inoculated with *Fusarium* sp. showed some infection. The spread of infection from the point of inoculation in no case extended more than 2.5 cm. on either side of the wound. In 4 plants of the 10 the infection had penetrated to the side opposite from the wound, thus girdling the root. Some softening of the tissues in the immediate vicinity of the wound was noted but no progressive rotting up and down the root was evident. In plants first injured by low temperature the roots deteriorated in 2 or 3 weeks.

Six plants inoculated with *Alternaria* sp. showed some infections, while the other 4 remained entirely healthy. In the 6 plants showing infection the discoloration penetrated only to a depth of 3–4 mm. and extended for about 1.25 cm. on each side of the wound. No softening of the tissues or progressive rotting of the roots was evident in any of the plants.

The other organisms used for inoculation failed to infect any of the plants and remained perfectly healthy in storage. All control plants that were incised but not inoculated remained healthy.

These results indicate that species of *Fusarium* are probably weak parasites of black locust, unable to seriously parasitize healthy plants but able to infect and cause root decay to injured plants as by freezing or by severe wounding. It is not unreasonable, therefore, to ascribe a secondary role to these fungi in the rotting of the roots of deciduous nursery stock in overwinter storage.

## DISCUSSION

The role of sub-freezing temperatures in predisposing nursery plants to root rot in overwinter storage has been shown in the preceding text. The storage work described for Iowa and Minnesota was repeated during the 1939–40 season with strikingly similar results. Cases of root rot in temporary field storage were traced repeatedly to unwitting exposures of the plants to temperatures below freezing as by leaving lifted plants overnight without adequate protection or leaving undercut stock in the nursery overnight, to shipping plants long distances in cold weather in plain unheated railway cars, or to faulty heel-in practices that permit mass movements of cold air within the heel-in trench as by using shingletow, sawdust, coarse sand, or insufficiently thick layers of soil in the heel-in trenches. A service memorandum issued to action agencies in the field warning of the danger of lifting stock in sub-freezing temperatures and of storing stock in improper storage resulted in elimination of practically all storage losses from root rot for 3 consecutive years where previously losses were experienced every year.

It has been noted earlier that losses of nursery stock at the Iowa and Minnesota nurseries have been repeatedly serious, while at the Missouri

nursery losses were seldom appreciable. Climate and local weather largely account for these differences. At the Missouri nursery only occasional severe temperatures occur before January and such spells of very cold weather are brief and transitory. At the Minnesota nursery, on the other hand, low temperatures may set in as early as the beginning of October. The nursery in Iowa is approximately midway, geographically, between the Minnesota and Missouri nurseries. Climatically it is much "nearer" the Minnesota nursery. At the Missouri nursery the plants for overwinter storage are lifted in December; at the Minnesota nursery, early in November; at the Iowa nursery, throughout November. A comparison of temperatures during periods of plant digging at these 3 nurseries showed that in Iowa freezing or sub-freezing temperatures prevailed in 8 of the 20 days required to lift the stock of black locust, or 40 per cent of the time; in Minnesota four days of the twelve, or 33 per cent of the time; while in Missouri the stock was lifted in 10 days in the month of December, and in every one of them mild temperatures prevailed. In view of these data, the weather factor explains why storage root rots in Iowa and Minnesota have been consistently serious, while in Missouri they have enjoyed considerable freedom from such troubles.

The conclusion is drawn that freezing injury has been the primary cause of major root deteriorations in storage of nursery seedlings in nurseries of the Upper Mississippi Valley. Fungi, of course, especially species of *Fusarium,* are essential to the destructive processes in root decay; but these activities must be classed as secondary in importance.

Differences in the relative resistance to low temperatures of the various species of plants used in these experiments are to be noted. Plants of black locust, Siberian elm, Osage orange, Russian mulberry, and wild plum apparently are the least resistant; while plants of Siberian pea tree and green ash, especially the latter, will withstand exposures to lower temperatures and for longer periods without serious injury.

## SUMMARY AND RECOMMENDATIONS

Rather heavy losses of nursery seedling stock have been under observation at 3 nurseries in the Upper Mississippi Valley. It was found that freezing injury to the roots at the time of lifting, in transit, or in inadequate storage have been primarily responsible for root deteriorations in storage. The roots of several species of plants may be injured by short exposures to temperatures only a few degrees below freezing. Nursery practices that may lead to injury of nursery stock if carried out under sub-freezing temperatures are: lifting of stock, leaving undercut stock in loosened ground, transporting or other outdoor handling of unprotected stock, storing plants in outdoor heel-in beds without adequate protection to the roots from low temperatures (as when stored in sawdust, shingletow, coarse sand, or shallow soil covering), and storing plants indoors in poorly insulated structures that fail to keep temperatures above freezing in the coldest periods. In storage,

plants thus injured were found invaded by numerous fungi the most common of which were species of *Fusarium* and *Alternaria*. In wound inoculations *Fusarium* produced small local lesions on the roots of healthy plants but none of the organisms used caused extensive decay. Adherence to tentative recommendations formulated as a result of this work has reduced storage losses to a negligible figure in nurseries where there had previously been frequent losses.

As far as practicable deciduous nursery stock should be lifted and handled when the temperature is above freezing. This policy should apply especially to the following species: black locust, Siberian elm, Osage orange, black walnut, Russian mulberry, and tulip tree. If lifting of stock must be done at temperatures below freezing, it should be confined to species such as green ash or Siberian pea tree, which are more resistant to low temperature.

For outdoor storage in the northern nurseries, deep heeling-in is important. Loamy soils are preferred for this purpose to insure good root protection in the trench and good drainage. Avoid heeling-in stock in porous media as shingletow, sawdust, or coarse sand in regions where winters are severe. As a sanitation measure, heel-in sites should preferably be rotated from year to year.

Storage facilities such as packing sheds, root cellars, and caves, where temperature fluctuations are controlled within narrow limits above freezing (34° to 50° F.), and where a high relative humidity is maintained, appear to be safe for storing most deciduous nursery stock over winter.

Care should be exercised in lifting and handling nursery stock so as not to injure the roots any more than is absolutely necessary, since wounds open the way for infections. In grading large quantities of nursery stock all plants that show severe mechanical injuries to the roots or that show lesions, molds, and other types of root infection should be discarded.

When nursery stock, especially of the frost-susceptible species, is shipped in bales or crates it should not be placed outdoors in improvised shacks, in unheated nursery buildings, or in unheated railway buildings during freezing weather; and the planting operations themselves should not be done in such weather. Weather Bureau information can be used as a guide in shipping to avoid exposing nursery stock to sub-freezing temperatures while in transit.

MISSISSIPPI AGRICULTURAL EXPERIMENT STATION,
STATE COLLEGE, MISS.

# HISTOLOGICAL STUDIES OF INFECTIONS OF THE COTTON HYPOCOTYL BY GLOMERELLA GOSSYPII AND FUSARIUM MONILIFORME

THOMAS J. HARROLD[1]

(Accepted for publication December 21, 1942)

## INTRODUCTION

Although several reports have been made concerning the pathogenicity of *Glomerella gossypii* (South.) Edg. and of *Fusarium moniliforme* Sheld. (1, 2, 3, 4, 5, 6, 7, 8) in cotton seedlings, apparently none has dealt with the problem from the histological point of view. It is the intent of this study to present histological evidence concerning the pathogenicity of the two organisms.

## MATERIALS AND METHODS

Acid-delinted seeds of *Gossypium hirsutum* variety, "College No. 1," were immersed for 10 minutes in a 1–1000 aqueous solution of $HgCl_2$, washed with sterile water, and dried. They were then divided into 3 lots; one lot being plated directly on water agar to serve as a check, the second lot was thoroughly mixed in a spore and mycelium suspension of *Glomerella gossypii* derived from a single-spore isolate and plated on water agar, the third lot was mixed with a similar suspension of *Fusarium moniliforme* derived from a single-spore isolate and plated as above. The isolates from seedlings to provide the inoculum used in this experiment, had previously been shown by T. J. Ratcliff to be virulent strains (unpublished data). Each lot consisted of 80 seeds plated in 20 Petri dishes containing 4 seeds each. The incubation temperature was 27° C. Fixations, in formal-acetic-alcohol, consisting of the seedlings from 2 plates from each group, were made daily for 8 days beginning on the second day following inoculation. Serial paraffin sections of a thickness of 20 μ, stained with Johansen's quadruple stain, were used.

## RESULTS

Germination proceeded rapidly under the conditions of the experiment. As a result, the hypocotyls of most of the seedlings in all of the groups extended ½ to ¾ inch beyond the seed coat on the second day after inoculation. The hypocotyls of the seedlings of the check group continued to elongate until at the conclusion of the experiment (10 days), they had attained lengths of 3 to 5 inches. No lateral roots were formed and no evidence of infection was observed on any of the check seedlings.

Mycelium of *Glomerella gossypii* was found growing on the agar on the second day after plating. During the course of the experiment the hypocotyls infected with this fungus attained lengths of little more than 1 inch,

[1] The writer is indebted to George E. Thompson for the single-spore isolations, to T. J. Ratcliff for assistance in the growth of the seedlings, and to O. K. Fletcher, Jr., for the photomicrographs.

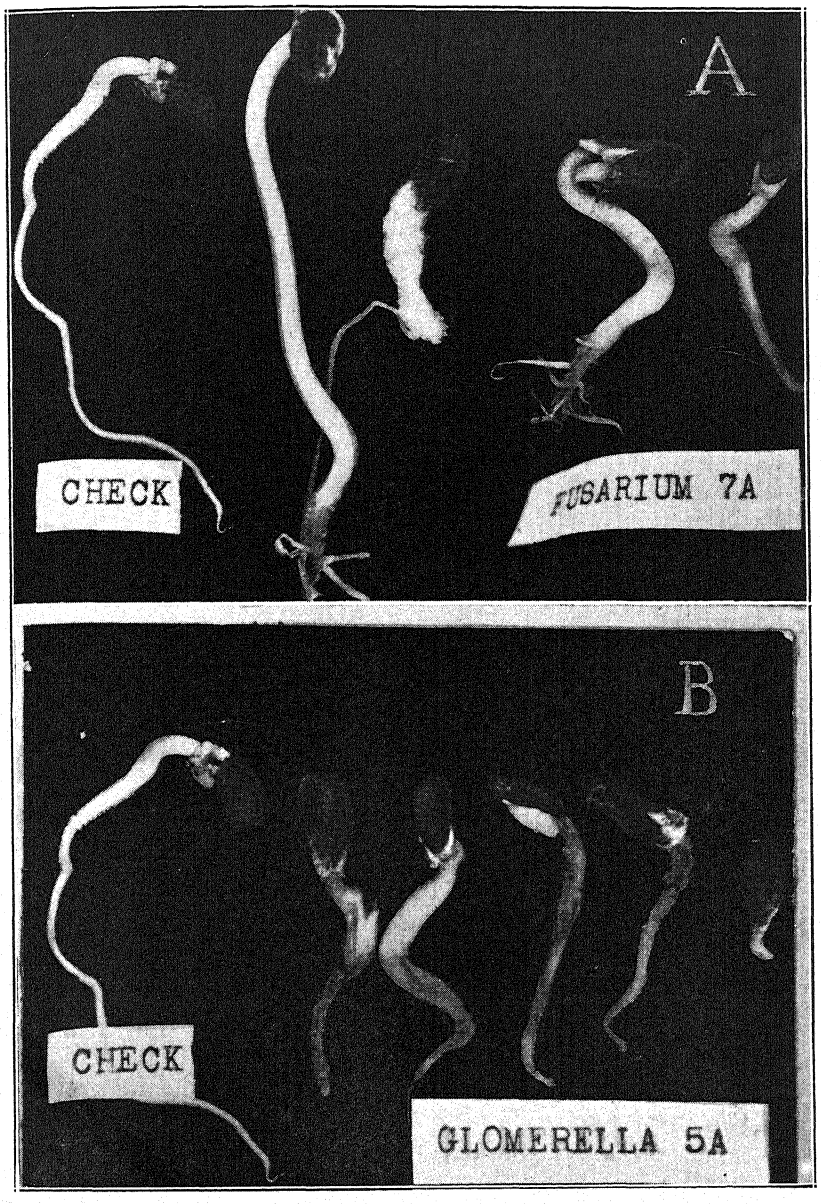



FIG. 1. Diseased cotton seedlings. A. Seedlings inoculated with *Fusarium moniliforme*, showing formation of lateral roots. B. Seedlings inoculated with *Glomerella gossypii*. ×1.25.

with an enlargement in diameter above the lesion and a constriction and apparent dying below that point (Fig. 1, B). No lateral roots were found on any of the hypocotyls infected by *G. gossypii*. Evidence of infection was found in cross sections of hypocotyls fixed on the fourth day after plating. At that time the mycelium was closely appressed to the surface of the hypo-

cotyl, was within root hairs and other epidermal cells, and also was within the parenchyma cells of the cortex, with certain individual strands extending nearly to the stele (Fig. 3, A, B). A flattening of the surface of the hypocotyl was noted at the apparent point of infection (Fig. 3, B). The mycelium spread rapidly in the horizontal plane within the cortex and into the stele. Strands of mycelium were observed wrapped around nuclei.

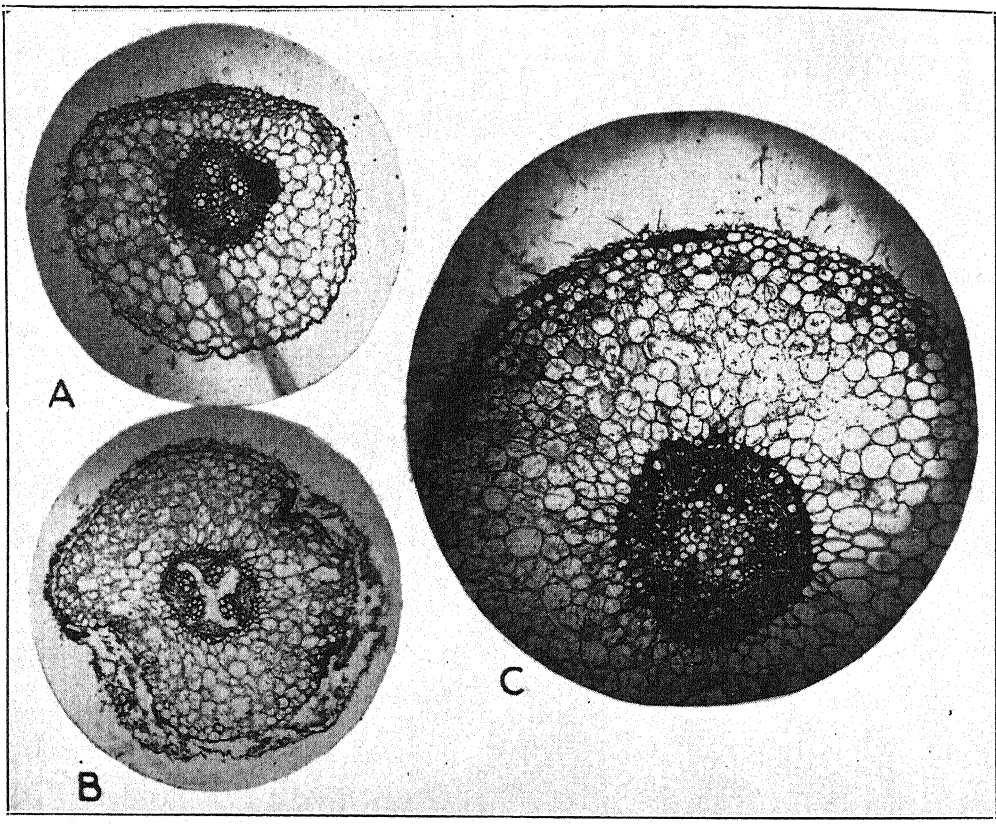



FIG. 2. Cross sections through cotton hypocotyls showing stages in the advance of the mycelium of *Fusarium moniliforme* within the hypocotyl. A. Early stage showing flattening of the side and the initiation of a lateral root near the point of infection. B. Later stage showing mycelium throughout cortex and within the stele and forming halo surrounding the hypocotyl. C. Intermediate stage showing localized mycelium penetrating as far as the stele. ×50.

Early stages of infection by *Glomerella gossypii* resulted in little or no apparent injury to individual cortical cells, (Fig. 3, A, B), while later stages show an apparent shrinking and indications of necrosis (Fig. 4, A, B; 5, B). In certain cases the infection remained localized, affecting only one side (Fig. 5, A), while in others the entire cross section of the hypocotyl was infected (Figs. 4, B; 5, B). Mycelium was found in the stele after considerable damage had been done to the cells of the cortex (Fig 5, B). All of the later stages revealed a halo of rather heavy external mycelium surrounding the hypocotyl on which acervuli were developing (Figs. 4, A, B; 5, A, B).

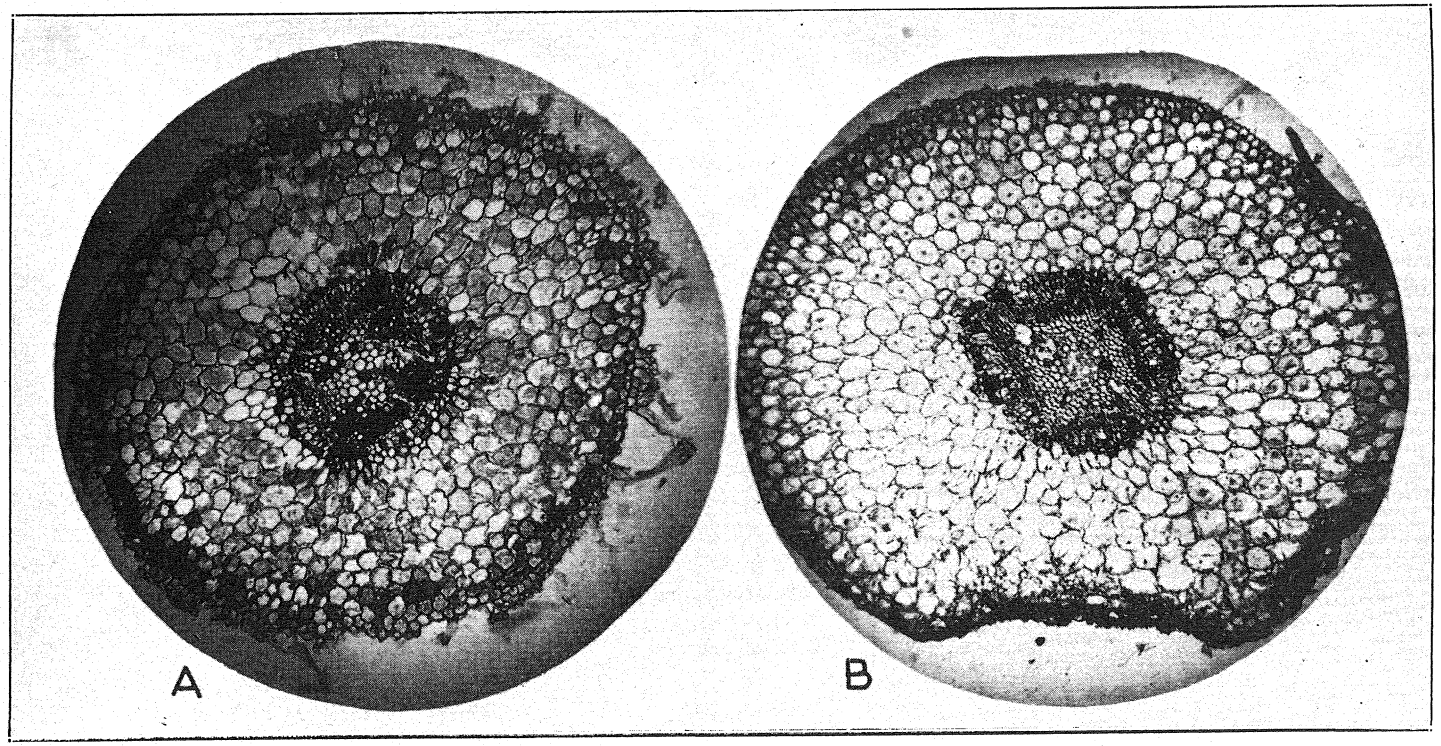



FIG. 3. Cross sections through cotton hypocotyls showing stages of penetration by *Glomerella gossypii*. A. Small amount of mycelium in upper, lower and left sides of section of hypocotyl. B. Flattening of side of hypocotyl at point of infection, individual strands of mycelium penetrating most of distance to the stele. ×50.

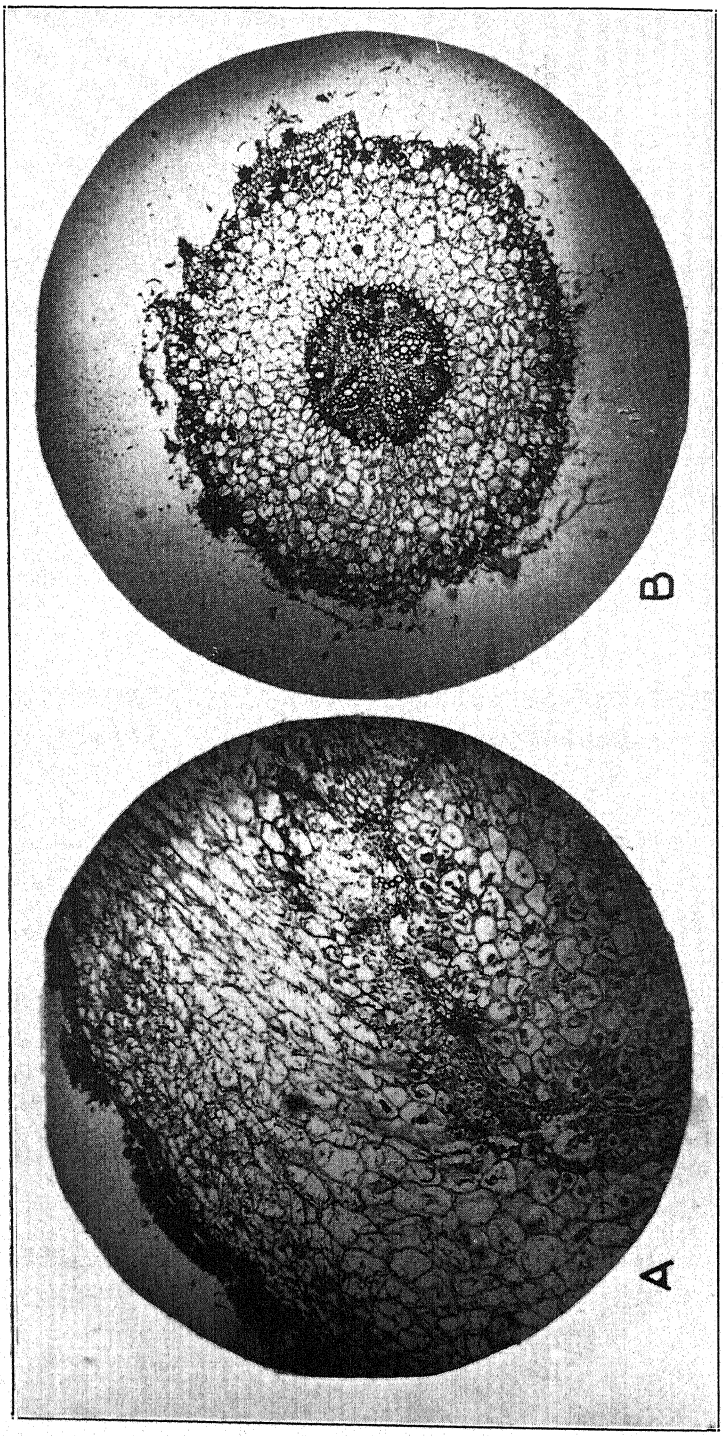



FIG. 4. Cross sections of hypocotyls infected by *Glomerella gossypii*. A. Heavy infection within cortical cells and acervuli upon the surface. B. Later stage, showing some shrinkage of cells, small amounts of acervuli and penetration of cortex and stele by the mycelium. ×50.

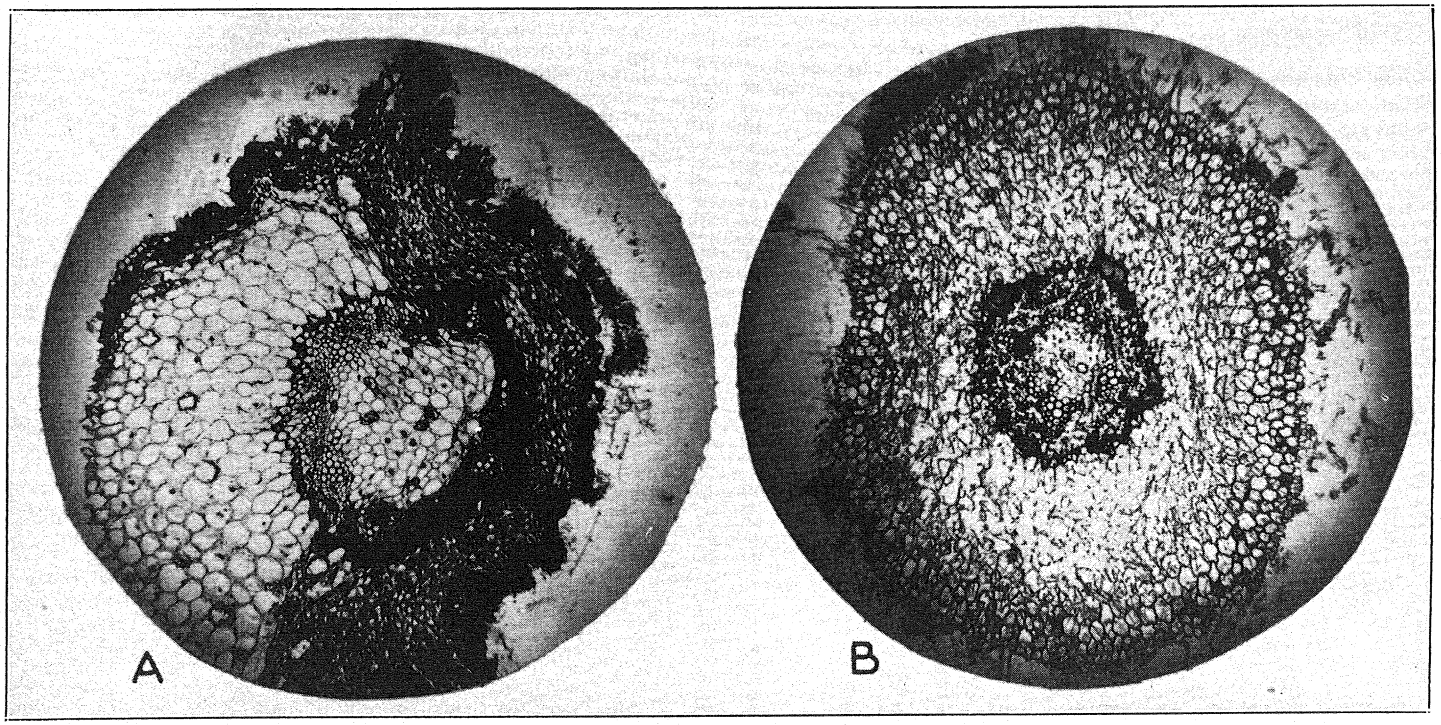



FIG. 5. Cross sections of cotton hypocotyls in late stages of infection by *Glomerella gossypii*. A. One side heavily infected, other side clear of infection. B. General penetration of mycelium throughout cortex and stele, with acervuli upon the surface and showing evident necrosis. ×50.

Infection of the cotton seedlings by *Fusarium moniliforme* followed much the same course as that described for *Glomerella gossypii*, as regards the time of appearance upon the agar, the entrance, and growth of the organism within the cells of the host (Fig. 2, A, B, C). The presence of the mycelium of *F. moniliforme* resulted in a similar effect on the cells of the cortex and stele, in a similar enlargement of diameter of the hypocotyl above the region of infection (Fig. 1, A), and in the flattening of the side of the hypocotyl at the point of infection (Fig. 2, A).

There were, however, several points of difference between the effects of the two organisms. The hypocotyls infected by *Fusarium moniliforme* in some cases attained lengths as great as the checks (5 inches), had less external mycelium, and were generally characterized by the appearance of a whorl of lateral roots at the region of infection (Figs. 1, A; 2, A).

## DISCUSSION

Since the mycelium of both *Glomerella gossypii* and *Fusarium moniliforme* apparently invaded the hypocotyl of cotton seedlings through root hairs and other epidermal cells, penetrated living cells of the cortex and stele and resulted in the death of these cells, it would appear that both organisms are primary intracellular parasites.

Infection by *Glomerella gossypii* has been reported by several writers as causing more damage to the cotton plant than does infection by *Fusarium moniliforme*. Arndt (1) found that *F. moniliforme* "will infect seedlings and produce stunting, but rarely death or damping-off of seedlings. Some parallel experiments with the anthracnose fungus under similar conditions showed 100 per cent killing, whereas there was 100 per cent survival with *F. moniliforme* though many of the seedlings were smaller than the disease-free controls." Arndt and Christie (2) reported that infection by *F. moniliforme* reduced the germination of cotton seed by 10 to 15 per cent, tended to increase the number of lesions on the hypocotyls, and to produce very irregular plants, but not to result in typical damping-off. *G. gossypii* caused typical damping-off in their experiments, killed 72 per cent of the plants and caused the surviving plants to be small and diseased.

Weindling *et al.* (7) noted that "*F. moniliforme* does not appear to be a primary pathogen of the damping-off of cotton seedlings," and in discussing their survey, found it "difficult at the present time to evaluate the high percentage of *F. moniliforme* obtained." These writers further stated that *G. gossypii* had been highly destructive in all cases. Woodroof (8) reported *F. moniliforme* as being the cause of stunting of cotton plants, but she did not believe that the organism entered living cells. The latter point is in variance with the findings of this study.

Ray and McLaughlin (5) reported both organisms as being pathogens of the cotton seedlings, *Glomerella gossypii* resulting in the more serious injury. Under conditions of excessive moisture these writers noted an increase in the relative pathogenicity of *Fusarium moniliforme* and with de-

creased moisture, lower temperature and increased alkalinity a relative increase in the pathogenicity of *G. gossypii*.

The differences in response of the cotton seedlings to infection by the two organisms cannot be explained on the basis of entrance and course of the mycelium within the cortex and stele of the hypocotyl. It may be inferred, although it is not demonstrated in this study, that differences in the spread of the organisms in the vertical plane might account for the differences in the effect on the host. Seedlings infected by *Fusarium moniliforme*, grown in Petri plates, attained in many cases normal or nearly normal lengths, while those infected by *G. gossypii* failed to elongate.

In the case of those seedlings infected by *Fusarium moniliforme* there was an appreciable tendency toward the initiation of lateral roots at the margin of the infected area, a condition totally absent, in this experiment, in the anthracnose-infected seedlings. A possible inference is that the initiation of the whorl of lateral roots is stimulated by some substance produced by the mycelium of *F. moniliforme* as it grows within the cells of the cortex.

## SUMMARY

Infections by *Glomerella gossypii* or by *Fusarium moniliforme* in cotton seedlings, in Petri plates, behaved similarly as regards penetration and course of growth within the host. Both are evidently intracellular parasites, which eventually induce death of the host cells. The greater tendency for survival following infection by *F. moniliforme* may possibly be accounted for by the stimulation of the production of lateral roots.

DEPARTMENT OF BOTANY,
UNIVERSITY OF GEORGIA,
ATHENS, GEORGIA.

## LITERATURE CITED

1. ARNDT, C. H. Laboratory studies with the anthracnose fungus, *Glomerella gossypii* and other organisms. S. C. Agr. Exp. Stat. Ann. Rept. 48: 41–42. 1935.
2. ———— and J. R. CHRISTIE. The comparative role of certain nematodes and fungi in the etiology of damping-off or soreshin of cotton. Phytopath. 27: 569–572. 1937.
3. BARRE, H. W. Cotton anthracnose investigation. S. C. Agr. Exp. Stat. Ann. Rept. 22: 89–118. 1909.
4. EDGERTON, C. W. The rots of the cotton boll. La. Agr. Exp. Stat. Bull. 13. 1912.
5. RAY, W. W., and J. H. MCLAUGHLIN Isolation and infection tests with seed- and soil-borne cotton pathogens. Phytopath. 32: 233–238. 1942.
6. ULLSTRUP, A. J. Variability of *Glomerella gossypii*. Phytopath. 28: 787–798. 1939.
7. WEINDLING, R., P. R. MILLER, and A. J. ULLSTRUP. Fungi associated with diseases of cotton seedlings and bolls with special consideration of *Glomerella gossypii*. Phytopath. 31: 158–167. 1941.
8. WOODROOF, N. C. A disease of cotton roots produced by *Fusarium moniliforme* Sheld. Phytopath. 17: 227–238. 1927.

# THE SENSITIVITY OF PLANT VIRUSES TO CERTAIN INACTIVATORS[1]

ROBERT W. FULTON

(Accepted for publication December 12, 1942)

## INTRODUCTION

In previous publications from this laboratory the inactivating effects of certain chemical substances upon plant viruses have been investigated, (1, 9, 10, 14). Particular interest has developed in the effect of several so-called "non-toxic" inactivators. A "non-toxic" inactivator is here meant to include a substance not detrimental to most forms of life, but one that does inactivate plant viruses. These include certain plant extracts, milk, trypsin, normal blood serum, and other complex substances of biological origin.

Most investigations of "non-toxic" inactivators have been concerned with but a single virus, namely, the ordinary tobacco-mosaic virus. Since other plant viruses have proved much more sensitive to toxic chemicals than has tobacco-mosaic virus (1), it appeared that some significant information might be secured by comparing the action of "non-toxic" substances upon these different viruses. Although it may be assumed that the nature of the reactions of viruses to toxic substances differs from that of their response to "non-toxic" ones, the magnitude of this difference is not at all clear. The present investigation was undertaken with the hope that detailed data on the effects of "non-toxic" inactivators on several viruses might provide evidence of the nature of this type of inactivation. The results obtained show that viruses differ in their sensitivity to the "non-toxic" inactivators used, as they also have been shown by Allington (1) and others to differ in their sensitivity to toxic chemicals.

## LITERATURE REVIEW

The first description of the ability of a supposedly innocuous substance to inactivate the tobacco-mosaic virus was by Duggar and Armstrong (6) for the juice of *Phytolacca decandra*. Numerous other substances have since been found to inactivate this virus. These include certain other plant juices (7), trypsin (12), normal blood serum (11), juices of certain insects (3), milk (5), casein, albumen, peptone (4), growth products of several species of microorganisms (10), and possibly tannic acid (13). While most of these have been investigated in their relation only to the tobacco-mosaic virus, Stanley (12) reported that trypsin inactivated the viruses of cucumber mosaic, severe etch, and tobacco ring spot. Chester (5) reported that normal blood serum inactivated the viruses of tobacco ring spot and cucumber mosaic, and Black (3) found that extracts of clover leaf-hoppers inac-

[1] Supported in part by allotments from the University of Wisconsin Research Fund. The author wishes to express his thanks to Dr. James Johnson for helpful advice and criticism during the course of the work and preparation of the manuscript.

tivated the viruses of potato yellow dwarf, potato X, turnip mosaic, tobacco necrosis, and tobacco ring spot. Johnson (9) reported that both milk and *Aerobacter aerogenes* growth product inactivated a number of viruses, including those of cucumber mosaic, potato veinbanding, and potato ring spot.

While there has been a lack of agreement as to the nature of the effect of these inactivators, certain features of their action are characteristic for the group, and have been observed repeatedly. For nearly all the substances investigated, inactivation has been observed immediately on mixing with virus (2, 3, 5, 7, 9, 12). Reactivation of the virus following removal or destruction of the inactivator has been reported also for all the "non-toxic" inactivators (5, 9, 12), except Phytolacca extract.

The immediate effect of these inactivators and the temporary nature of that effect have led to the belief that the virus itself is not affected, but rather that the inoculated host is rendered less susceptible (5, 12). The principal proof advanced by Stanley (12) in favor of this view is based on a test in which a series of virus dilutions are mixed with constant amounts of inactivator, and the percentage inactivation determined by inoculation to a host yielding local lesions. A greater percentage reduction of local lesions with the more dilute extracts is accepted as indicating an effect on the virus, while a similar percentage reduction at all dilutions is accepted as indicating an effect on the host. Results of this test, particularly with trypsin and blood serum, have been interpreted differently by different investigators (4, 5, 12, 15).

Evidence also has been presented indicating that certain inactivators affect the virus. Hills and Vinson (8) showed that the rate of diffusion of tobacco-mosaic virus particles was slowed by the presence of trypsin, and concluded that the virus and the trypsin combined. Johnson (9) has shown that for many toxic chemicals a period of contact with the virus is necessary for maximum inactivation. This method of proof cannot be used with the "non-toxic" inactivators, which exert their full effect immediately on mixing with virus. It was hoped that the present investigation might yield additional evidence as to whether the host or the virus was affected, or that it might suggest methods of obtaining such evidence.

## METHODS AND MATERIALS

The following 5 "non-toxic" substances were selected as representative of this class of inactivators: *Phytolacca decandra* L. extract (stored under toluene at 4° C.), trypsin (Difco, standardized), fresh cow's milk, bovine serum (stored frozen), and *Aspergillus niger* van Tiegh. growth product (the liquid from a two weeks broth culture evaporated to 1/10 original volume, stored frozen). The viruses used were those of tobacco mosaic (*tobacco virus 1*), tobacco ring spot, cucumber mosaic (*cucumber virus 1*), potato ring spot, and bean mosaic. Young Havana Seed No. 38 tobacco plants were used in tests for the first 4 viruses, and Wisconsin Refugee Stringless bean seedlings for the bean-mosaic virus.

While the local-lesion method is rapid and relatively accurate for determining inactivation, it could not be used conveniently with several of the viruses. Therefore, in order to make the results as comparable as possible, inactivation of all viruses was determined by inoculating 5 young healthy plants in each test, and recording the number that became infected as a measure of the degree of inactivation. Five such tests, involving a total of 25 plants for each concentration of inactivator, were made for each mixture. It is recognized that this method determines only the concentration at which inactivation is complete, or very nearly so.

To provide inoculum, young, recently infected plants were ground up, the juice filtered through cheese cloth and then diluted. In preliminary tests it was found necessary to dilute the tobacco-mosaic virus more than the other viruses in order to obtain a demonstrable degree of inactivation. Similarly some of the inactivators were less potent; this necessitated using greater dilutions of all the viruses. All inoculations were made within 2 or 3 minutes after mixing the virus and the inactivator.

## EXPERIMENTAL RESULTS

### Relative Sensitivity of the Viruses to Inactivation

As illustrative of the type of results obtained, the detailed data on 2 viruses and 2 inactivators are presented in table 1. The results obtained on the inactivation of all 5 viruses with the 5 inactivators are summarized in table 2. From the data in this table the 5 viruses may be arranged in the order of their sensitivity to the inactivators as a group. Thus, bean-mosaic virus usually required the lowest concentration for complete inactivation,

TABLE 1.—*Representative data showing the amount of inactivation induced by various concentrations of two inactivators on two viruses diluted to 1:1000*

| Inactivator | Virus of | Percentage concentration of inactivators and number of plants infected out of 25 inoculated | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 80 | 70 | 60 | 50 | 40 | 30 | 25 | 20 | 15 | 10 | 5 | 0 |
| Bovine serum | Tobacco ring spot | ..... | ..... | ..... | ..... | ..... | 0 | 0 | 1 | 3 | 8 | 16 | 25 |
| | Cucumber mosaic | ..... | ..... | ..... | ..... | ..... | 0 | 3 | 5 | 6 | 9 | 14 | 25 |
| Aspergillus growth product | Tobacco ring spot | 0 | 3 | 2 | 3 | 9 | ..... | ..... | 13 | ..... | ..... | ..... | 25 |
| | Cucumber mosaic | ..... | ..... | ..... | ..... | 0 | 3 | ..... | 9 | 11 | 13 | 18 | 25 |

followed by tobacco-ring-spot virus, cucumber-mosaic virus, potato-ring-spot virus, and tobacco-mosaic virus, in that order. This same order of sensitivity for the latter 4 viruses also was found by Allington (1) when toxic chemicals were used. He found that the cucumber mosaic and potato-ring-spot viruses did not differ greatly in their sensitivity to the toxic chemicals. In the present investigation, however, using "non-toxic" inactivators, the

potato-ring-spot virus was definitely more tolerant than the cucumber mosaic virus.

There were, however, some marked variations in the order of sensitivity of the viruses to certain inactivators. The tobacco-ring-spot virus, for example, is inactivated by a lower concentration of bovine serum than is the cucumber-mosaic virus. The latter, however, is inactivated by a lower concentration of *Aspergillus* growth product than is the tobacco-ring-spot virus (Table 1). Similarly, a higher concentration of Phytolacca extract is necessary to inactivate the bean-mosaic virus than is required for the tobacco-

TABLE 2.—*Summary showing concentrations of 5 inactivators required to inactivate 5 different viruses. (Each figure based on 5 tests of 5 plants each)*

| Inactivator | Concentration[a] | Viruses, at dilutions shown, and percentage concentration of inactivator required | | | | |
|---|---|---|---|---|---|---|
| | | Bean mosaic 1:10 | Tobacco ring spot 1:100 | Cucumber mosaic 1:100 | Potato ring spot 1:100 | Tobacco mosaic 1:1000 |
| Phytolacca juice | A | 0.25 | 0.2 | 1.25 | 3.0 | 5.0 |
| | B | 0.125 | 0.1 | 1.0 | 2.5 | 4.0 |
| Milk | A | 7.5 | 25.0 | 20.0 | ..... | ..... |
| | B | 5.0 | 20.0 | 15.0 | 99.0[b] | 99.0[b] |
| Trypsin | A | 0.25 | 1.0 | 3.0 | 3.5 | ..... |
| | B | 0.125 | 0.5 | 2.5 | 3.0 | 3.5[b] |
| | | Bean mosaic 1:10 | Tobacco ring spot 1:1000 | Cucumber mosaic 1:1000 | Potato ring spot 1:1000 | Tobacco mosaic 1:10,000 |
| Bovine serum | A | 10 | 25 | 30 | ..... | ..... |
| | B | 5 | 20 | 25 | 99[b] | 99[c] |
| Aspergillus growth product | A | ..... | 80 | 40 | ..... | ..... |
| | B | 90[b] | 70 | 30 | 99[c] | 99[c] |

[a] A—Lowest percentage concentration resulting in complete inactivation.
B—Highest percentage concentration not resulting in complete inactivation.
[b] One to 5 plants infected out of 25 inoculated.
[c] Ten to 20 plants infected out of 25 inoculated.

ring-spot virus; whereas considerably higher concentrations of the other inactivators are required for the tobacco-ring-spot virus. The actual amount of virus present was undoubtedly different in the original preparations of different viruses. The relative concentrations of the viruses, however, were constant in the trials with the different inactivators. The results indicate, therefore, that properties inherent to the virus affect its sensitivity to "non-toxic" inactivators, as has been demonstrated previously for toxic chemicals (1).

## The Effect of Virus Concentration on Inactivation

The results in table 2 seemed to indicate that the concentrations of the viruses influenced to some degree the concentration of inactivator required. Tobacco-mosaic and potato-ring-spot viruses, which required the highest con-

centrations of the inactivators, exist in high concentrations in infected tobacco plants, whereas the viruses of cucumber mosaic and tobacco ring spot exist in much lower concentrations. In comparing the effectiveness of a single inactivator on different viruses the relative infectivities of the virus preparations should be considered. The concentrations of the viruses in the extracts were compared, therefore, by means of dilution tests. Five plants were inoculated in each of 5 trials with a given dilution (Table 3). It is difficult to compare the viruses because of the differences in the shape of the dilution curves. Infectivity of the extracts of tobacco-ring-spot virus dropped sharply on dilution, while those of cucumber-mosaic virus, over

TABLE 3.—*Relative infectivities of 5 viruses as determined by their tolerance to dilution, with the same method of inoculation used in all trials*

| Virus of | Dilution and number of plants infected out of 25 inoculated | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 : 30 | 1 : $10^2$ | 1 : $3 \times 10^2$ | 1 : $10^3$ | 1 : $3 \times 10^3$ | 1 : $10^4$ | 1 : $3 \times 10^4$ | 1 : $10^5$ | 1 : $3 \times 10^5$ | 1 : $10^6$ | 1 : $3 \times 10^6$ | 1 : $10^7$ | 1 : $3 \times 10^7$ | 1 : $10^8$ | 1 : $3 \times 10^8$ |
| Bean mosaic ... | 24 | 18 | 5 | 4 | 2 | 0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Tobacco ring spot | ..... | ..... | ..... | 25 | 21 | 10 | 0 | 0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Cucumber mosaic ... | ..... | ..... | ..... | ..... | 9 | 9 | 5 | 2 | 1 | 0 | ..... | ..... | ..... | ..... | ..... |
| Potato ring spot | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 15 | 6 | 5 | 4 | 0 | 0 | ..... |
| Tobacco mosaic ... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 22 | 14 | 1 | 1 | 0 | 0 |

much the same range, lost infectivity much more gradually on dilution. Comparisons are thus only approximate. If the infectivity of the bean-mosaic virus extracts is taken as 1 the relative infectivities of the virus extracts of tobacco ring spot, cucumber mosaic, potato ring spot, and tobacco mosaic are about 30, 100, 10,000, and 30,000, respectively.

The virus extracts of bean mosaic, tobacco ring spot, and cucumber mosaic, as used in inactivation trials, were nearly equal in infectivity. In general, the lowest concentrations of inactivator were required for the bean-mosaic-virus extracts, which were also the least infective. Higher percentages of inactivator were usually required for the more infective virus extracts of tobacco ring spot and cucumber mosaic. Less inactivator was required, however, for the viruses of potato ring spot and tobacco mosaic than would be expected on the basis of their greater infectivities. For example, 3.0 per cent trypsin is required to inactivate the cucumber-mosaic virus at a dilution of 1 : 100, and 3.5 per cent trypsin is required for potato-ring-spot virus at the same dilution, yet the infectivity of the latter virus was about 100 times as great as the former. When different dilutions of viruses are mixed with an inactivator some of this effect might be due to the higher concentration of extraneous materials in the least dilute extract interfering with the activity of the inactivator. This should not be im-

portant, however, in the case of extracts tested at the same dilution. Either the potato-ring-spot and tobacco-mosaic viruses are more susceptible to all the inactivators, or, since they were used at higher concentrations than the others, their concentration may have been responsible for their inactivation by relatively low percentages of inactivators.

As a means of determining the effect of virus concentration on inactivation 3 dilutions of tobacco-mosaic virus were mixed with 3 dilutions of inactivator, keeping the ratio of virus to inactivator the same at each dilution. These mixtures were inoculated to the *Nicotiana tabacum* × *N. glutinosa* hybrid leaves, yielding local lesions, in a 3 × 3 Latin square arrangement, with the mixture on one-half of a leaf, and the corresponding dilution of virus alone on the other half. The results with 4 inactivators are presented in table 4. With this virus the inactivation percentage decreased as the concentration of the mixture became lower. It seems likely, therefore, that the greater infectivities of the extracts of tobacco-mosaic

TABLE 4.—*The relation of the concentration of mixtures of the tobacco-mosaic virus and inactivators to the amount of inactivation resulting*

| Mixture | | | Total number of lesions on test plants produced by | | |
|---|---|---|---|---|---|
| Virus concentration | Inactivator and concentration | | Virus plus inactivator | Control (virus only) | Percentage reduction due to inactivator |
| | | *Per cent* | | | |
| 1: 500 | Trypsin | 0.4 | 27 | 1139 | 97.6 |
| 1: 2,500 | " | 0.08 | 55 | 178 | 68.7 |
| 1: 12,500 | " | 0.016 | 48 | 95 | 49.5 |
| 1: 500 | Milk | 8 | 14 | 1020 | 98.6 |
| 1: 2,500 | " | 1.6 | 25 | 501 | 95.1 |
| 1: 12,500 | " | 0.32 | 27 | 96 | 71.9 |
| 1: 500 | Phyt. juice | 0.15 | 44 | 1920 | 97.7 |
| 1: 2,500 | " " | 0.03 | 62 | 821 | 92.5 |
| 1: 12,500 | " " | 0.006 | 73 | 219 | 67.7 |
| 1: 500 | Blood serum | 8 | 25 | 1467 | 98.3 |
| 1: 2,500 | " " | 1.6 | 88 | 294 | 70.1 |
| 1: 12,500 | " " | 0.32 | 71 | 72 | 1.4 |

and potato-ring-spot viruses resulted in their being inactivated by proportionately lower concentrations of inactivators than the more dilute viruses. The reactivating effect of dilution on mixtures of some of these inactivators has been reported (5, 12).

An interesting, and probably significant, observation on the effect of dilution is the difference in degree to which it affects the different inactivators. The inactivation with milk dropped only from 98 to 72 per cent on dilution. On the other hand, diluting bovine serum mixtures to the same extent decreased the inactivation from 98 per cent to practically no inactivation. These results seem best explained on the basis of a difference in tenacity of combination between virus and inactivator.

## DISCUSSION

An accurate comparison of the sensitivities of viruses to a particular inactivator probably would require the use of purified virus, purified inactivator, and virus preparations with the same concentration of infective particles. Since these requirements could not be met in the present investigation, an attempt was made to secure relative data by comparing the sensitivities of several viruses to several inactivators. It is, for example, of little significance to determine that the tobacco-ring-spot virus is inactivated by a lower concentration of bovine serum than the cucumber-mosaic virus, since the actual amount of virus being inactivated in each case is not known. If in addition, however, the same dilution of cucumber-mosaic virus is inactivated by a lower concentration of *Aspergillus* growth product than the tobacco-ring-spot virus, then a difference between the sensitivities of these two viruses may be assumed. Several such differences between the viruses of bean mosaic, tobacco ring spot, and cucumber mosaic were demonstrated. These differences seem to be further evidence that the effect of "non-toxic" substances is on the virus itself, and not on the host.

The results of diluting mixtures of inactivator and virus also throw some doubt on the validity of dilution tests used to determine whether a substance affects the virus or the host. This test assumes that, if the virus is affected, a definite amount of inactivator will act upon a definite amount of virus regardless of the relative concentrations of the two. On the basis of the results presented in table 4, however, mixtures of a high virus concentration and a definite amount of inactivator may be expected to show a larger proportion of the virus inactivated than of mixtures with a lower virus concentration. This is essentially the result obtained with trypsin in dilution tests involving mixing constant concentrations of inactivator with different dilutions of virus, and that has been interpreted as being due to an effect of trypsin on the host. When the effect of dilution on an inactivator is relatively large, results would be obtained indicating that the effect of the inactivator is on the host. It may be significant in this connection that there is a relatively great drop in percentage of inactivation when mixtures of trypsin or blood serum are diluted. Both these substances have been described as examples of "inhibitors" that do not act on the virus.

The results obtained offer no evidence contradictory to the idea, as expressed by Thornberry (13), that this type of inactivation may be due to a reversible interaction between inactivator particles and virus particles. Earlier work has shown that, except for Phytolacca extracts, inactivation is reversed when the inactivator is removed or destroyed. In connection with the present investigation it has been found that virus mixtures with Phytolacca extract may also be reactivated by the proper technique.[2] All the

[2] Virus extracts of potato ring spot, cucumber mosaic, tobacco ring spot, and tobacco mosaic were each mixed, as in table 2, with the lowest percentage of Phytolacca extract necessary for complete inactivation. Talc was then added at the rate of 2 g. per 50 cc. of mixture, shaken thoroughly, then allowed to settle. The decanted liquid yielded 100

"non-toxic" inactivators used, therefore, seem to behave similarly in this respect.

While the nature of the effect of an inactivator on a virus is not known, some evidence suggests that a reversible adsorption may be concerned. This conception need not be entirely incompatible with the evidence pertaining to the reactivation of virus by ultra filtration, ultra centrifugation, or diffusion techniques. Such a possibility should, at least, be considered before attributing the principal effect of "non-toxic" inactivators to a reduction of host susceptibility.

## SUMMARY

The sensitivity of viruses to inactivation by trypsin, milk, extract of *Phytolacca decandra,* bovine serum, and *Aspergillus niger* growth product was determined. The viruses used were those of tobacco mosaic, potato ring spot, cucumber mosaic, tobacco ring spot, and bean mosaic. In general, the sensitivity of the viruses to the inactivators increased in the order named above. The viruses of bean mosaic, tobacco ring spot, and cucumber mosaic, however, exhibited specific responses to the extent that the order of sensitivity to certain inactivators was reversed. The virus extracts of tobacco mosaic and potato ring spot were much more concentrated than the other virus extracts, but required only moderately higher concentrations of inactivators. The percentage of tobacco-mosaic virus inactivated was greatest when the mixture with inactivator was most concentrated, least when the mixture was most dilute. As a whole, the results supported the view that the effect of the "non-toxic" inactivators is on the virus and not on the host.

DEPARTMENT OF HORTICULTURE, UNIVERSITY OF WISCONSIN,
MADISON, WIS.

## LITERATURE CITED

1. ALLINGTON, W. B. The separation of plant viruses by chemical means. Phytopath. 28: 902–918. 1938.
2. BAWDEN, F. C., and N. W. PIRIE. Experiments on the chemical behavior of potato virus "X." Brit. Jour. Exp. Path. 17: 64–74. 1936.
3. BLACK, L. M. Inhibition of virus activity by insect juices. Phytopath. 29: 321–337. 1939.
4. CALDWELL, JOHN. Factors affecting the formation of local lesions by tobacco-mosaic virus. Proc. Roy. Soc. (London) (B) 119: 493–506. 1936.
5. CHESTER, K. STARR. Specific quantitative neutralization of the viruses of tobacco mosaic, tobacco ring spot, and cucumber mosaic by immune sera. Phytopath. 24: 1180–1202. 1934.
6. DUGGAR, B. M., and J. K. ARMSTRONG. The effect of treating the virus of tobacco mosaic with the juices of various plants. Ann. Mo. Bot. Gard. 12: 359–366. 1925.
7. GRANT, T. J. The host range and behavior of the ordinary tobacco-mosaic virus. Phytopath. 24: 311–336. 1934.
8. HILLS, C. H., and C. G. VINSON. Particle size of tobacco-mosaic virus. Mo. Agr. Exp. Stat. Res. Bull. 286. 1938.
9. JOHNSON, JAMES. Chemical inactivation and the reactivation of a plant virus. Phytopath. 31: 679–701. 1941.

per cent infection in each case, whereas mixtures not treated with talc produced no infection. Using the local-lesion method with the tobacco-mosaic virus it was noted that the reactivation was not necessarily always complete.

10. JOHNSON, JAMES, and ISMÉ, A. HOGGAN. The inactivation of the ordinary tobacco-mosaic virus by microorganisms. Phytopath. 27: 1014–1027. 1937.
11. MULVANIA, M. Studies on the nature of the virus of tobacco mosaic. Phytopath. 16: 853–871. 1926.
12. STANLEY, W. M. Chemical studies on the virus of tobacco mosaic. I. Some effects of trypsin. Phytopath. 24: 1055–1085. 1934.
13. THORNBERRY, H. H. Effect of tannic acid on the infectivity of tobacco-mosaic virus. Phytopath. 25: 931–937. 1935.
14. WENT, JOHANNA C. The influence of various chemicals on the inactivation of *tobacco virus 1*. Phytopath. Zeitschr. 10: 480–489. 1937.
15. YOUDEN, W. J., H. P. BEALE, and J. D. GUTHRIE. Relation of virus concentration to the number of lesions produced. Contrib. Boyce Thomp. Inst. Plant Res. 7: 37–53. 1935.

# ORGANIC MATERIALS IN PRE-HARVEST SPRAYS FOR CHERRIES

D. H. PALMITER AND J. M. HAMILTON[1]

(Accepted for publication November 24, 1942).

The standard practice of applying sulphur sprays and dusts for controlling the brown rot, *Sclerotinia fructicola,* Wint. Rhem., and gray mold, *Botrytis cinerea* Pers., of sweet cherries has not been entirely effective. Irrespective of control, the various sulphur treatments have one serious defect: They all leave noticeable deposits of spray residue. And spray residues of any nature are highly objectionable to fruit consumers.

Since 1938, the writers have been trying to find some material, other than sulphur, that could control brown rot of cherries, without visible residue on the fruit. They reported[2] promising results from the use of an extremely dilute red copper oxide spray, at ¼ lb. in 100 gal. of water, plus a spreader. This spray, however, causes copper injury which necessitates an after-harvest lime spray to prevent serious leaf drop. To overcome the undesirable properties of the sulphur and copper fungicides, recent investigations were conducted to test certain of the most promising organic materials.

## MATERIALS TESTED

Three of the new organic materials, recently shown to be toxic to fungi at low concentrations, were tested as pre-harvest applications on sweet cherries. They were ferric-dimethyl-dithio-carbamate (Fermate), tetra-methyl-thiuram-disulphide (Jap Beetle Spray), and tetrachloro-para-benzoquinone (Spergon).[3] Micronized sulphur was used as the standard treatment. S. E. C. Oil (self-emulsifiable cottonseed oil) was employed as a spreader and sticker in comparative tests to determine whether it enhanced control of the very dilute fungicide applications that were necessarily employed to avoid residues.

## DISEASE CONTROL

### Spore Germination Experiments

*Methods.* In conjunction with the field control tests, spore germination tests[4] were made to determine the relative toxicity of the Jap Beetle Spray and Fermate materials in comparison with sulphur and red copper oxide. The glass slides used in these tests were coated with cellulose nitrate. The slides were exposed to a given spray for 15-, 60-, and 120-second intervals.[5]

[1] Approved by the director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 532, November 7, 1942.

[2] Parrott, P. J. New York Agr. Exp. Sta. Ann. Rpt. 1938: 24. 1939.

[3] Fermate and Jap Beetle Spray were supplied by duPont de Nemours Co. Spergon was furnished by the U. S. Rubber Co. Red copper oxide and S. E. C. Cottonseed Oil were from Röhm & Haas.

[4] Horsfall, J. G., J. W. Heuberger, E. G. Sharvelle, and J. M. Hamilton. A design for laboratory assay of fungicides. Phytopath. 30: 545–563. 1940.

[5] Hamilton, J. M., and G. L. Mack. A new vertical laboratory sprayer (in preparation).

One half of the slides for each treatment were subjected to washing by passing the slides 30 times through distilled water after the spray had thoroughly dried.[6] The other half were left unwashed. Each of the 4 fungicides was used alone and with S. E. C. Oil, 1–1600. Three uniform drops of a spore suspension of *Sclerotinia fructicola,* adjusted to a concentration of 60 spores per sq. mm. in a Fuchs-Rosenthahl counting cell, were placed on each slide. After incubation in moist chambers at a temperature favorable for germination, 100 spores were counted from each of 3 drops and averaged for the percentage of spore inhibition.

TABLE 1.—*Comparative toxicity of organic and inorganic fungicides to spores of Sclerotinia fructicola on coated glass slides*[a]

| Treatment | Grams per liter | Seconds exposed to spray | Ave. percentage of spores inhibited from germinating | |
|---|---|---|---|---|
| | | | Unwashed slides | Washed slides |
| Untreated | ..... | ..... | 3.5 | 3.0 |
| Micronized sulphur | 6.0 | 15 | 78.5 | 5.5 |
| | | 60 | 91.0 | 26.0 |
| | | 120 | 100.0 | 82.0 |
| Micronized sulphur + S. E. C. Oil[b] | 6.0 | 15 | 73.5 | 53.0 |
| | | 60 | 100.0 | 86.0 |
| | | 120 | 100.0 | 100.0 |
| Red copper oxide | 2.0 | 15 | 25.5 | 25.5 |
| | | 60 | 44.5 | 45.0 |
| | | 120 | 76.5 | 75.5 |
| Red copper oxide + S. E. C. Oil | 2.0 | 15 | 26.0 | 26.0 |
| | | 60 | 43.5 | 40.5 |
| | | 120 | 61.0 | ..... |
| Jap Beetle Spray | 2.5 | 15 | 57.5 | 18.0 |
| | | 60 | 100.0 | 56.0 |
| | | 120 | 100.0 | 70.0 |
| Jap Beetle Spray + S. E. C. Oil | 2.5 | 15 | 59.5 | 58.0 |
| | | 60 | 100.0 | 100.0 |
| | | 120 | 100.0 | 100.0 |
| Fermate | 2.5 | 15 | 76.0 | 79.5 |
| | | 60 | 90.0 | 88.5 |
| | | 120 | 100.0 | 100.0 |
| Fermate + S. E. C. Oil | 2.5 | 15 | 82.0 | 80.5 |
| | | 60 | 97.5 | 94.5 |
| | | 120 | 100.0 | 100.0 |

[a] The glass slides were coated with nitro-cellulose acetate.
[b] S. E. C. Oil is a self-emulsifying cottonseed oil. It was used at the rate of 1–1600.

*Results.* Fermate and the Jap Beetle Spray were about equally toxic on unwashed slides, whereas red copper oxide and micronized sulphur were only $\frac{1}{3}$ to $\frac{1}{2}$ as potent (Table 1). On washed slides, the spore counts show that the micronized sulphur spray deposit was reduced in effectiveness to a greater extent than any of the other materials. The Jap Beetle Spray too lost considerable effectiveness when washed. In contrast, Fermate and the red copper oxide showed only small differences in toxicity between the

[6] Heuberger, John W. A laboratory biological assay of tenacity of fungicides. Phytopath. 30: 840–847. 1940.

washed and unwashed slides. Loss of toxicity following washing is interpreted as due to partial removal of the original spray deposits.

The addition of S. E. C. Oil significantly increased the retention of the Jap Beetle Spray and of the sulphur, and made for an inhibitory residue equivalent to that of the unwashed slides. In the case of the Fermate and red copper oxide, however, the use of S. E. C. Oil made little difference in their ability to inhibit germination.

## Orchard Tests

*Methods.* All the sprays were applied with a power sprayer to mature orchard trees, of Windsor, Schmidt, Black Republican, Black Tartarian, and Giant varieties. The trees had received the following spray schedule: A dormant application of two per cent tar oil; four applications of flotation sulphur paste at 6–100 (6 pounds in 100 gallons of water), at the green-tip, petal-fall, shuck-split, and shuck-fall stages (the latter two sprays also contained arsenate of lead for curculio); two cover sprays for cherry fruit fly control, the first consisting of micronized sulphur, 4–100, plus rotenone, and the second of micronized sulphur, 2–100, plus rotenone and one-half pint of S. E. C. Oil.

Fermate, Jap Beetle Spray, and Spergon were tested at two concentrations: ½–100 on fruit about ready for harvest and 1–100 on fruit which was to remain on the trees for a longer period of time. The first pre-harvest spray was applied June 13. Micronized sulphur, 2–100, was used as a standard of comparison. S. E. C. Oil, ½ pint to 100 gallons, was used with all the fungicides.

## Disease Control on Fruit at Time of Picking

*Results.* Fruits from Schmidt, Giant, and Black Republican cherry trees, sprayed with the Fermate and the Jap Beetle Spray had not over 5 per cent rot 2 weeks after treatment (Table 2). Most of this decay, especially on the Giant variety, was the result of infections on cracked fruits. Rotted fruit on the sulphur-sprayed trees ranged from 19 per cent on the

TABLE 2.—*Effectiveness of sulphur and organic materials in pre-harvest sprays for controlling brown rot and gray mold on sweet cherries*

| Variety | Pre-harvest treatment[a] | Percentage of fruit rotted 2 weeks after treatment[b] |
|---|---|---|
| Schmidt | Micronized sulphur 2–100 | 19 |
| | Fermate 1–100 | 2 |
| | Jap Beetle Spray 1–100 | 0 |
| Giant | Micronized sulphur 2–100 | 38 |
| | Fermate 1–100 | 5 |
| | Jap Beetle Spray 1–100 | 2 |
| | Spergon 1–100 | 33 |
| Black Republican | Fermate 1–100 | 0 |
| | Jap Beetle Spray 1–100 | 0 |

[a] S. E. C. Oil, one-half pint to 100 gallons, was included in all treatments.
[b] A total of 4.07 inches of rain fell between the pre-harvest application and the time the results were taken.

Schmidt variety to 38 per cent on the Giant. Spergon gave no better control than the sulphur. The nontreated cherries in each variety were completely diseased.

Much of the rot that developed on the early varieties was gray mold caused by *Botrytis cinerea,* which developed during the 2-week interval between spray and harvest in which 4 inches of rain furnished favorable conditions for rot development.

Additional data on the value of the Jap Beetle Spray were obtained from an application of this material, ½–100, plus ½ pint of S. E. C. Oil to a block of Windsor trees (a later variety) that had received the pre-harvest spray of micronized sulphur, 2–100, 3 weeks earlier when the other varieties were

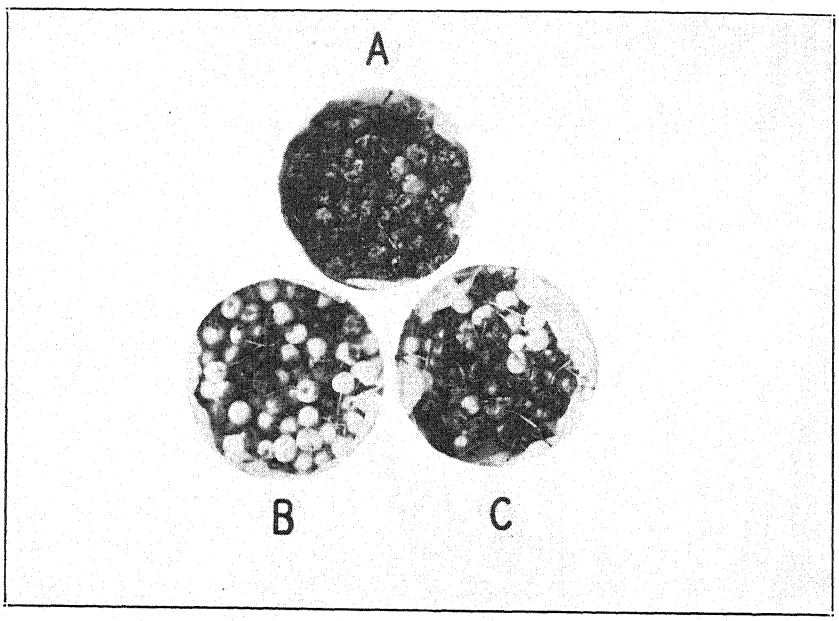



FIG. 1. Brown-rot infection on Napoleon cherries after 8 days in a moist chamber at room temperature following immersion in a spore suspension of *Sclerotinia fructicola:* A. No spray; B. Fermate ½–100; C. Micronized sulphur 2½–100.

sprayed. The fruit was picked a week after treatment during which time almost two inches of rain fell. This additional spray reduced the percentage of rotted fruit from 9.4 to 2.7.

Field tests in 1942 substantiated the data of 1941. One pre-harvest application of Fermate, ½–100, plus ½ pint of S. E. C. Oil, to Napoleon cherries one week before harvest lowered the percentage of rot from 92 per cent, as on nonsprayed trees, to 52 per cent. Two applications of the Fermate, the above and one 17 days earlier, reduced the amount of rot at harvest to 18 per cent; whereas a similar plot receiving two applications of micronized sulphur, 5–100 and 2½–100, respectively, had 41 per cent of the fruit infected with brown rot (Fig. 1). The comparatively high percentage of decay in these plots is no doubt due to the long interval between applications during which 2. 23 inches of rain fell.

The data from two seasons' field trials show that the degree of superiority of Fermate over the standard sulphur spray in the pre-harvest application depends on the number and thoroughness of the previous applications and the environmental conditions.

## Disease Control on Fruit After Picking

Although the sweet cherry may leave the orchard apparently sound, serious loss from brown rot and gray mold often develops during the period of transportation and marketing. Since the pre-harvest spray treatment affects their keeping quality, tests were made to compare the effectiveness of the different materials in this regard.

Samples of sound fruits, taken from the various pre-harvest spray plots, were immersed in a spore suspension of *Sclerotinia fructicola* or *Botrytis cinerea*. This inoculated fruit was then placed in moist chambers, consisting of 600-ml. beakers lined with moist paper towels and covered with waxed paper. They were held for incubation periods of 3 to 6 days at room temperature (75°–85° F.).

*Protection Against Brown Rot.* Fruit from Black Tartarian trees, which had just been sprayed with Fermate, Jap Beetle Spray, and Spergon, ½–100, plus S. E. C. Oil (½ pint–100), was practically all sound after 3 days in the moist chamber, while the unsprayed fruit was 38 per cent rotted (Table 3, Ser. 1). After 6 days, the unsprayed fruits were all infected, while the sprayed fruits showed only from 24 to 32 per cent decay.

The Windsor trees, which had received the late pre-harvest treatment of Jap Beetle Spray, ½–100, plus S. E. C. Oil, had 17 per cent of their fruits decayed after 3 days in the moist chamber, as contrasted with 49 per cent for the check (Table 3, Ser. 2). This count included fruits that had cracked by water-absorption in the moist chambers, which opened an avenue for infection regardless of the spray treatment.

Fermate, 1–100, applied to Schmidt, Giant, and Black Republican trees two weeks before picking, held the percentage of rotted fruit after 6 days in the moist chambers to a low level, when compared with fruits from trees receiving sulphur, 2–100, or no pre-harvest treatment (Table 3, Ser. 3). This heavier dosage of the organic fungicide significantly improved the protection of the inoculated fruit through the post-harvest moist treatment (Table 3, Ser. 1 and Ser. 3). The fruits on this block had been washed by 4 inches of rain before they were picked. Attention is called to these results particularly, because it furnishes an excellent illustration of the ability of the Fermate to resist washing.

*Protection Against Gray Mold.* Fruits treated with Fermate and Jap Beetle Spray, 1–100, or micronized sulphur, 2–100, developed practically no gray mold after 3 days in the moist chamber, whereas the Spergon-sprayed fruits at 1–100 and the nontreated fruits showed 22 and 36 per cent decayed fruits, respectively (Table 4). S. E. C. Oil (½ pt.–100) was used with all treatments.

At the end of 6 days, Fermate was the most effective material with not

TABLE 3.—*Effectiveness of sulphur and organic materials in pre-harvest sprays in preventing after-harvest brown rot infections on sweet cherries*[a]

| Series and variety | Pre-harvest treatment[b] | Percentage of fruit rotted after incubation period of | |
|---|---|---|---|
| | | 3 days | 6 days |
| Series 1[c] | | | |
| Black Tartarian | Nontreated | 38 | 100 |
| " | Fermate ½–100 | 2 | 24 |
| " | Jap Beetle Spray ½–100 | 0 | 24 |
| " | Spergon ½–100 | 2 | 32 |
| Series 2[d] | | | |
| Windsor | Nontreated | 49 | ..... |
| " | Jap Beetle Spray ½–100 | 17 | ..... |
| Series 3[e] | | | |
| Windsor | Nontreated | ..... | 100 |
| Schmidt | Micronized sulphur 2–100 | ..... | 64 |
| Giant | Micronzied sulphur 2–100 | ..... | 54 |
| Schmidt | Fermate 1–100 | ..... | 5 |
| Giant | Fermate 1–100 | ..... | 0 |
| Black Republican | Fermate 1–100 | ..... | 11 |

[a] Picked fruits were inoculated with spores of *Sclerotinia fructicola* and kept in moist chambers.

[b] One-half pint of S. E. C. Cottonseed Oil was included in all pre-harvest sprays.

[c] Fruit in this series was picked after a light shower the same day it was sprayed.

[d] This treatment was made a week before harvest and the fruit was washed by two inches of rain during that interval.

[e] These treatments were made two weeks before harvest and the fruit was washed by 4.07 inches of rain during that interval.

over 6 per cent of the fruit infected that reflects greater retention during washing. The Jap Beetle Spray and sulphur were about equally effective, allowing 18 per cent of the fruit of the Giant variety to become decayed. The fruits treated with Spergon and the nontreated were 76 and 94 per cent infected, respectively.

TABLE 4.—*Effectivness of sulphur and organic materials in pre-harvest sprays in preventing after-harvest gray mold infections on sweet cherries*[a]

| Variety | Pre-harvest treatment[b,c] | Per cent of fruit rotted after incubation period of | |
|---|---|---|---|
| | | 3 days | 6 days |
| Windsor | Unsprayed | 36 | 94 |
| Schmidt | Micronized sulphur 2–100 | 4 | 8 |
| Giant | Micronized sulphur 2–100 | 2 | 18 |
| Schmidt | Jap Beetle Spray 1–100 | 0 | 2 |
| Giant | Jap Beetle Spray 1–100 | 4 | 18 |
| Republican | Jap Bettle Spray 1–100 | 0 | 8 |
| Schmidt | Fermate 1–100 | 0 | 0 |
| Giant | Fermate 1–100 | 0 | 4 |
| Republican | Fermate 1–100 | 2 | 6 |
| Giant | Spergon 1–100 | 22 | 76 |

[a] Picked fruits were artificially inoculated with spores of *Botrytis cinerea* and kept in moist chambers.

[b] One-half pint of S. E. C. Oil was included in all pre-harvest sprays.

[c] These treatments were made two weeks before harvest and the fruit was washed by 4.07 inches of rain during that interval.

### FRUIT CRACKING

Sweet cherries treated with sprays containing self-emulsifying cottonseed oil were observed to have less cracking than those that were unsprayed (Table 5). The reduction in cracking is attributed to the cottonseed oil.

TABLE 5.—*Effectiveness of Jap Beetle Spray and S. E. C. Oil in reducing brown rot and cracking of cherries*[a]

| Variety | Additional pre-harvest treatment | No. of fruits counted | Percentage of fruit cracked | Percentage of fruit rotted |
|---|---|---|---|---|
| Windsor | Nontreated | 300 | 14.7 | 9.4 |
| Windsor | Jap Beetle Spray ½–100, plus ½ pint S. E. C. Oil | 474 | 5.5 | 2.7 |

[a] This fruit was washed by 1.97 inches of rain between the pre-harvest spray and harvest.

The presence of this oil in the various sprays seemed to form an invisible coating over the fruit, that did not permit absorption of rain water. Oil-sprayed cherries which were immersed in water after picking cracked just

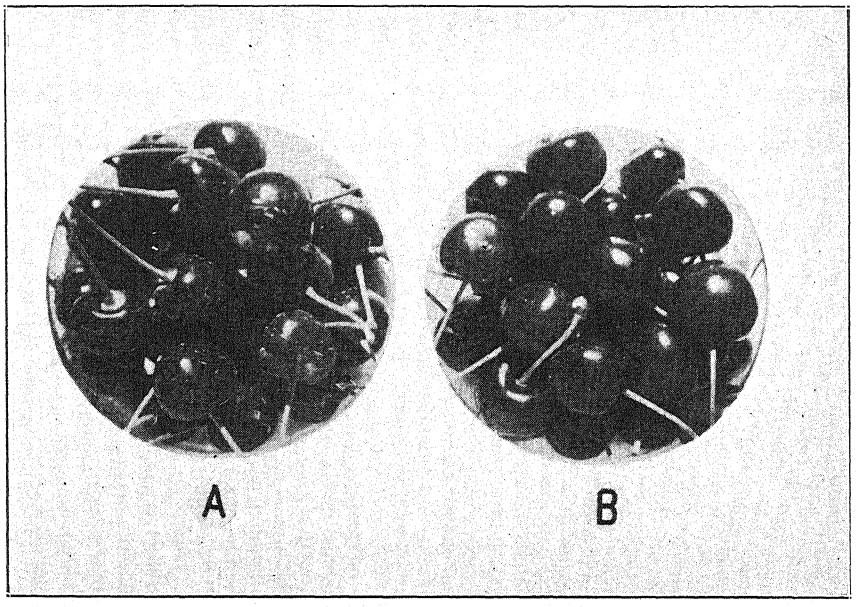



FIG. 2. Comparison of visible residue on Schmidt cherries after spraying with: A. Micronized sulphur 2½–100 plus SS–3 1½ ounces. B. Fermate 1–100 plus S. E. C. Oil ½ pint.

as much as the unsprayed fruits, indicating that the oil functions only to shed the rain.

### RESIDUES

Data from the seasons of 1941 and 1942 indicate that Fermate, 1–100, and Jap Beetle Spray, 1–100, are the most effective and desirable of the pre-harvest fungicides tested.

Fermate used alone leaves a black, spotty coverage on cherry fruits, but when a suitable spreader such as S. E. C. Oil, ½ pint, is used with it, the residue is not objectionable on any of the dark varieties of cherries (Fig. 2). On white varieties, Fermate can be used with satisfactory results up to the final pre-harvest application at which time Jap Beetle Spray, 1–100, or wettable sulphur, 2½–100, with a suitable spreader-sticker, may be substituted to avoid the dark residue on the light fruit. Under certain conditions, as where growers with mixed varieties wish to make frequent applications to protect each variety as it reaches maturity, it may be possible and desirable to reduce the concentration of Fermate to ½–100 with the expectation of good results.

## SUMMARY

The organic materials, Fermate and Jap Beetle Spray, when sprayed on cellulose-nitrate-coated glass slides were found to be equally toxic against the spores of *Sclerotinia fructicola*. These two materials exhibited two to three times greater toxicity than micronized sulphur or red copper oxide, which have been used for controlling brown rot on sweet cherries. Washing of the sprayed slides before applying the spores indicates the greater retention of the Fermate and red copper oxide over that of Jap Beetle Spray or sulphur. The addition of soluble cottonseed oil markedly increased the amount of toxic residue retained by Jap Beetle Spray and sulphur-sprayed slides.

Fermate and Jap Beetle Spray were superior to Spergon and micronized sulphur when tested as pre-harvest sprays on sweet cherries for control of brown rot and gray mold. The data indicate that the Fermate and Jap Beetle Spray should be used at 1–100, a concentration which could be expected to give appreciable leaf-spot control. These materials at ½–100 have been used with success when more than one application is made or when applied during the harvest period.

Moist-chamber studies and observations made in the market show that Fermate used as a pre-harvest spray enhanced the keeping quality of the cherries on the fresh fruit market more than any of the other materials tested.

Fermate to which a good spreader has been added avoids the objectionable visible residue caused by sulphur sprays on dark colored cherries, but this dark spray material shows more than Jap Beetle Spray or sulphur on light colored fruit.

An emulsified vegetable oil has been found a desirable addition to the pre-harvest spray both from the standpoint of rendering the residue less visible and reducing the amount of cracking.

NEW YORK STATE AGRICULTURAL EXPERIMENT STATION,
GENEVA, NEW YORK.

# A TENDENCY TO ESCAPE TOBACCO-MOSAIC DISEASE IN DERIVATIVES FROM A HYBRID TOMATO

FRANCIS O. HOLMES

(Accepted for publication January 27, 1943)

The Chilean tomato, *Lycopersicon chilense* Dun.,[1] became available as a potential source of resistance to diseases of tomato in 1937, through discovery of a viable seed in an approximately 12-year-old herbarium specimen (3). Studies were begun at once to determine its susceptibility to tobacco mosaic, a disease to which all tested varieties and strains of the garden tomato, *L. esculentum* Mill., have proved susceptible and with which all have been readily infected by abrasive methods of inoculation. The results of these studies, briefly reported already in an abstract (4), have shown that the Chilean tomato is characterized by a tendency to escape infection and that this klendusity is of a heritable nature, being found also in certain derivatives of the hybrid *L. esculentum* × *L. chilense*. The purpose of this paper is to present in detail the evidence on which these conclusions are based.

## KLENDUSITY OF THE CHILEAN TOMATO AND SOME OF ITS HYBRID PROGENY

The Chilean tomato proved difficult to infect with tobacco-mosaic virus (*Marmor tabaci* H.). When once infected, however, it developed a systemic chlorotic-mottling type of disease.

The $F_1$ hybrid *Lycopersicon esculentum* ♀ × *L. chilense* ♂ (3), although intermediate between the parental species in general appearance and especially in fineness of leaf-cutting, was characterized by vigor of vegetative growth. The $F_1$ plants proved intermediate in susceptibility, escaping infection somewhat more often than ordinary tomato but much less often than *L. chilense*. When infected, they showed chlorotic mottling much like that developing in infected plants of the two parent species. Plants of this first hybrid generation were of low fertility. Their pollen, however, proved adequate to produce a backcross generation.

Plants of the backcross generation, *Lycopersicon esculentum* ♀ × (*L. esculentum* × *L. chilense*) ♂, were exceedingly variable in appearance, showing almost every conceivable combination of the foliage and fruit characteristics of the two original parental species. They were not inoculated with tobacco-mosaic virus, since it appeared probable from the manner of their origin that they would share the relatively high susceptibilities of the $F_1$ hybrid and of *L. esculentum*. Instead, they were permitted to set open-pollinated fruits in the greenhouse insofar as they were capable of doing so.

[1] In a recent revision of the genus *Lycopersicon* by Muller (U.S.D.A., Misc. Publ. 382) the Chilean tomato is treated as the variety *dentatum* (Dun.) Muller of *L. peruvianum* (L.) Mill. Pending confirmation of the indicated relationship, however, the writer prefers to consider it as a separate species. Typical representatives of the species *L. peruvianum* have not shown comparable facility of hybridization with *L. esculentum* thus far.

Some proved highly fertile, others less fertile, and a few appeared sterile. Seeds obtained from a number of the individuals were planted.

Some sets of seedlings secured in this way as segregating offspring from individual plants of the backcross ($B_1$) generation appeared on the whole much like the cultivated tomato; others showed characters obviously resembling those of *Lycopersicon chilense*. All were tested by inoculation with tobacco-mosaic virus. Unfamiliarity with the degree of klendusity to be anticipated in the segregating populations may have resulted in failure to detect what later would have been readily recognizable tendencies to escape disease in individuals in some of these populations. However that may be, only one individual capable of escaping disease in the manner of the Chilean tomato was identified. It greatly resembled the wild species in leaf shape but was more vigorous in growth and larger in size. It was the only seedling obtained from one particular plant of the first backcross generation.

This klendusic seedling did not fruit for some time. Finally, after about two years in the greenhouse, it produced and matured a considerable number of fruits as a result of open pollination. The fruits were a little larger than the $\frac{3}{8}$- to $\frac{1}{2}$-inch fruits of the Chilean parent, were marked with a much less conspicuous purple stripe, and displayed a yellow, rather than a cream, color when fully ripe. From 0 to 4 seeds were produced in each fruit; from the first 153 fruits exactly 153 well formed seeds were obtained and planted. From them 12 seedlings grew. Of these, 6 became infected in early tests; whether they were klendusic or not was not properly determined, adequate technique for determining klendusity not being available at the time. Of the remaining seedlings, 3 grew well enough to be available for more extensive tests. When their leaves were rubbed in the presence of juice expressed from mosaic tobacco leaves, after dilution such that ordinary tomato plants would generally but not always become infected in comparable tests, all 3 seedlings showed the tendency to escape infection that had been noted both in the Chilean tomato from which they had originated and in their immediate parent, the lone seedling from a backcross plant.

Identification of the tendency to escape infection in the parental generation and in two successive generations following hybridization and backcrossing appeared to be adequate proof of the heritability of this desirable characteristic. Its mode of inheritance could not be investigated at the time because of low fertility among available derivatives from the hybrid *Lycopersicon esculentum* × *L. chilense*.

### EXPERIMENTS WITH SEEDLING 1196C2

The effectiveness of the tendency to escape infection was tested under a variety of conditions in 1 of the 3 seedlings above mentioned. This plant, which will be referred to by its record number, 1196C2, was propagated for more than a year by rooting successive lots of tip cuttings. It differed strikingly from *Lycopersicon chilense* in its more vigorous growth and in

having foliage less finely divided than that of most varieties of the garden tomato, *L. esculentum*.

Plants of 1196C2 and of Bonny Best tomato, in all cases grown from cuttings, were inoculated by rubbing the tips of 3 leaves of each with successive dilutions of juice freshly expressed from a mosaic plant of Turkish tobacco, *Nicotiana tabacum* L. Figure 1 shows the results of this and a subsequent experiment. In the figure, individuals infected in this experiment are represented by the use of black symbols, circles for plants of 1196C2, and triangles for Bonny Best control plants. It will be observed that all plants of 1196C2 were infected in the test with undiluted juice, clearly showing the essential susceptibility of this clone of plants, but that few were infected by the 1 : 10 and none by the 1 : 100 or higher dilutions. On the other hand, the Bonny Best tomato plants were all infected in tests with undiluted juice and the 1 : 10 dilution, many by the 1 : 100 dilution, and some by the 1 : 1000 dilution. Had more leaf surface been rubbed on each plant, somewhat less concentrated inocula might have proved effective both for the ordinary tomato and for 1196C2 plants.

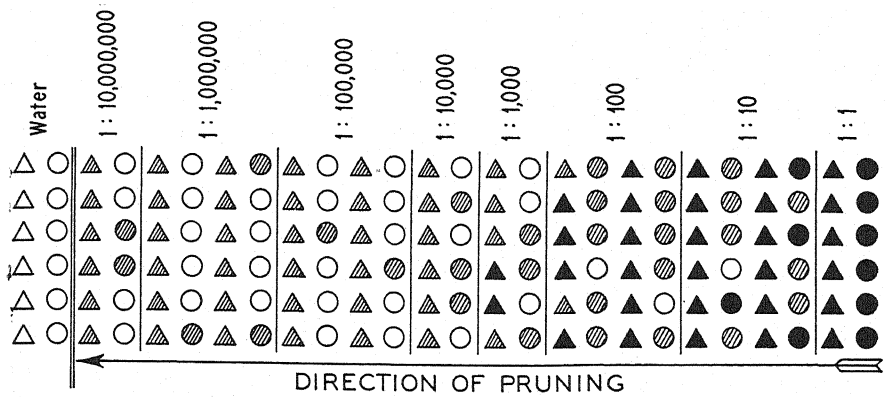



FIG. 1. Results of rubbing and subsequent pruning inoculations of Bonny Best plants (triangles) and 1196C2 plants (circles), as described in text. Black symbols indicate plants that became infected by the initial rubbing inoculation with the diluted juice samples specified. Shaded symbols indicate plants infected only by the subsequent pruning, which proceeded from right to left along each row (last two plants in each row not pruned). Unshaded symbols indicate plants that were not infected by either of these operations. Alternation of plant types on a greenhouse bench is indicated.

It was concluded from this experiment that samples of juice from mosaic plants of tobacco would be most serviceable as inocula for distinguishing klendusic from fully susceptible plants if diluted somewhat more than 1 : 10 but less than 1 : 100 with water. An intermediate dilution, 1 : 50, was tested and proved particularly suitable. It often infected none of the 1196C2 plants in a particular experiment but all of the Bonny Best plants used as controls. This dilution was used thereafter to identify klendusic plants.

A tendency to remain healthy after contaminative superficial contacts, such as those furnished by rubbing inoculation, would not necessarily imply resistance in the presence of other kinds of virus transfer. Cutting back of

vines and pruning of branches on successive diseased and healthy tomato plants constitute a type of inoculation commonly occurring as a result of field and greenhouse practices. These procedures involve wounding of tissues well below the surface of the plant. It was thought that if the klendusity of plant 1196C2 should be dependent on some peculiarity of surface cells alone, cutting operations might render the protective mechanism ineffective. To test this point, the plants earlier used for testing different concentrations of inoculum were all cut back to within 2 inches of the ground, beginning in each row at the end where heaviest inoculations had been applied and most infections had occurred and proceeding toward the end where all plants had escaped infection. Plants originally inoculated with water alone were not included and remained healthy throughout the course of the experiment. The plants to be pruned were cut successively with a razor blade that was cleaned by washing with soap and water only after the completion of each row. The blade was not cleaned during the cutting of successive plants within each row except as the cutting operation itself tended to wipe away virus obtained from diseased plants. The cutting instrument became thoroughly contaminated in removing the tops of the first few plants in each row and, in consequence of this, infected all of the Bonny Best tomato plants that it subsequently cut; nevertheless, more than half of the interspersed plants of 1196C2 escaped. This will be seen from the record presented in figure 1, in which plants infected by this pruning operation are indicated by shaded symbols. All of 42 newly exposed Bonny Best plants and 30 of 62 1196C2 plants were infected. If only exposed plants not immediately preceded by diseased individuals are considered, all of 42 Bonny Best tomato plants and only 8 of 36 1196C2 plants became infected. It may be concluded from this and the preceding experiment that the klendusity of 1196C2 plants was effective in the presence of both rubbing and cutting types of inoculation.

Tendency to escape infection from deep wounding operations suggested that the mechanism responsible for klendusity in 1196C2 plants was present in many and perhaps all cells of the plant and was not confined to superficial layers. The nature of this mechanism was not obvious, however. Some light was thrown on the problem by an observation that infected plants of 1196C2, although they became conspicuously mottled, served poorly as sources of inoculum for infection of *Nicotiana glutinosa* L. Transfers from 6 mosaic Bonny Best tomato plants to 6 plants of *N. glutinosa* resulted in an average of 1175 lesions per test plant; comparable transfers from 6 mosaic plants of 1196C2 yielded only 352 lesions per plant. In each case the source plants had been infected for 7 weeks and were showing distinct chlorotic mottling when used.

### DEMONSTRATION OF INHIBITOR

The coupling of conspicuous mottling with what appeared to be low yields of virus in infected plants of 1196C2 suggested that juices of the diseased

plants might be inhibitory rather than actually low in virus content. The question arose, therefore, whether the juices from healthy 1196C2 plants might tend to restrain formation of lesions on inoculated plants of *Nicotiana glutinosa* in the presence of virus from another source. That this was actually the case was shown by inoculating with 3 mixtures, each containing 1 part of juice expressed from mosaic Bonny Best tomato plants diluted with 9 parts of one of the following: water, healthy 1196C2 juice, and healthy Bonny Best tomato-plant juice. Each sample was tested by inoculating 3 plants of *N. glutinosa*. The mixture with water induced the formation of 264, 152, and 420 lesions, that with juice of 1196C2 plants 38, 40, and 38 lesions, and that with Bonny Best juice 285, 243, and 205 lesions. The striking reduction in numbers of lesions caused by using juice from healthy plants of 1196C2 demonstrated that some decidedly inhibitory agent was present in this juice. It suggested that the nature of the juice might have played an important part in preventing some 1196C2 plants from becoming infected in earlier experiments and might have accounted for the apparently low virus content of the individual plants that were infected. If juices of 1196C2 plants act in as inhibitory a way upon infection of their own tissues as upon infection in tests with *N. glutinosa*, it is unnecessary, perhaps, to seek further for a plausible explanation of klendusity in these plants, since it may be assumed safely that some juices are always liberated from an inoculated surface at the moment of inoculation. These juices may be sufficient to affect virus or infected cells, or both, in such a way as to prevent subsequent development of disease. It is possible, of course, that some klendusic plants later may be found not to possess an inhibitory juice. Should this be the case, another mechanism would have to be sought to explain the observed klendusity. Tentatively, however, the phenomenon may be considered as probably attributable to some inhibitory constituent or constituents of expressed juice.

It was thought that advantage from the inhibitory effect of 1196C2 juice might be greater under some circumstances than the inoculating and pruning experiments thus far described had indicated. Contaminative contacts under field conditions of tomato culture are likely to involve virus from within rather than from outside the planting. To test what might be expected to happen if klendusic plants could be substituted for ordinary types of tomatoes in field practice, a greenhouse experiment was devised, in which mosaic plants of 1196C2 and a succession of healthy individuals of the same clone were handled in a way simulating the handling contacts of plants taken from a seed bed for transplanting. For controls, diseased and healthy Bonny Best tomato plants were manipulated in a similar manner. Plants of each kind were arranged in sets of 6 healthy individuals, each set accompanied by a mosaic plant of its own type. For the first test, the right hand was used to grasp successively the infected 1196C2 plant and its 6 healthy counterparts, and simultaneously the left hand was used in the same way for Bonney Best controls. Then both hands were washed thoroughly with

soap and water and the process reversed to compensate for any differences in manner of making contacts with right and left hands. In the second test, the left hand was used for 1196C2 plants, the right hand for Bonny Best controls. The same thorough washing of hands and reversal of procedure was practiced before making the third and fourth tests. Results of this experiment on contaminative handling contacts were striking. None of 24 exposed 1196C2 plants, but all of 24 exposed Bonny Best plants, became infected. The klendusic tendencies possessed by this derivative from the hybrid *Lycopersicon esculentum* × *L. chilense* appeared likely to be highly effective under conditions resembling those in field plantings.

It cannot be predicted yet whether the characteristic of klendusity inherent in plant 1196C2 and its immediate relatives can be transferred to ordinary varieties of tomato, nor whether such transfer if accomplished would be compatible with high quality and yield of fruit. During the period of experimentation, plant 1196C2 has blossomed less abundantly than its immediate parent, which was also klendusic. It has not yet set fruit, though now more than a year old. Naturally it is hoped that sterility will not be complete, for this would require a return to plants of an earlier generation for the production of the additional and more fertile stocks needed to permit investigation of the mode of inheritance of klendusity.

## DISCUSSION

Klendusity with respect to inoculation through superficial abrasions and the types of injury involved in pruning operations obviously would not assure that there would be a tendency also to escape infection by insect feeding, should efficient vectors of tobacco-mosaic disease be encountered under field conditions. From time to time suspicion has rested on hypothetical insect vectors as possibly responsible for spread of tobacco-mosaic disease in tomatoes on certain farms where other detectable means of spread have seemed inadequate to account for the observed incidence. It seems probable, however, that any insect transmission that may exist will be difficult to demonstrate under field conditions until spread by mechanical means can be minimized or eliminated. Continued experimentation with klendusic derivatives from the *Lycopersicon esculentum* × *L. chilense* hybrid would seem to be justified even in the absence of knowledge of how such plants may be affected by insect transmission. Production of horticulturally useful klendusic varieties, if feasible, would constitute a step toward elucidation of the still nebulous insect-vector problem in connection with tobacco-mosaic disease in tomatoes in the field, all past experimental work on the subject with other than negative results having been performed under laboratory conditions (1, 2).

The tendency to escape disease encountered in *Lycopersicon chilense* and its derivatives stands in contrast with a trait that has been described as protective in *L. hirsutum* Humb. and Bonpl. This second protective characteristic, discovered by Porte, Doolittle, and Wellman (5), provides a

passive reaction to infection. It is characterized by no hindrance to infection, by high activity of juice from diseased plants, and by absence of obvious signs of disease. *L. chilense* and its klendusic derivatives present a very different picture, involving a tendency to escape infection, a low activity of juice from diseased plants, and clear symptoms of disease in any plants that become infected. *L. hirsutum* derivatives, if responding like the parent species, would escape damage themselves but would constitute a concealed reservoir of virus if infected. *L. chilense* derivatives would tend to remain healthy, *i.e.*, virus-free, but if infected would respond much as ordinary tomatoes do. Both mechanisms may prove valuable for reducing the often underestimated effects of tobacco-mosaic disease in commercial plantings of tomatoes.

SUMMARY

A heritable characteristic of klendusity with respect to abrasive and pruning types of inoculation with tobacco-mosaic virus has been found in the Chilean tomato, *Lycopersicon chilense* Dun., and in certain derivatives from its hybrid with the cultivated tomato, *L. esculentum* Mill. Whether this tendency to escape disease can be transferred to and incorporated in ordinary tomato varieties, and that without interfering with quality or yield of fruits, remains to be demonstrated.

DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY,
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

LITERATURE CITED

1. HOGGAN, I. A. Further studies on aphid transmission of plant viruses. Phytopath. 21: 199–212. 1931.
2. ———. Transmissibility by aphids of the tobacco mosaic virus from different hosts. Jour. Agr. Res. [U. S.] 49(1934): 1135–1142. 1935.
3. HOLMES, F. O. The Chilean tomato, *Lycopersicon chilense*, as a possible source of disease resistance. Phytopath. 29: 215–216. 1939.
4. ———. Derivatives of tomato that tend to escape tobacco-mosaic disease. Phytopath. (Abstract) 33: 19. 1943.
5. PORTE, W. S., S. P. DOOLITTLE, and F. L. WELLMAN. Hybridization of a mosaic-tolerant, wilt-resistant *Lycopersicon hirsutum* with *Lycopersicon esculentum*. Phytopath. 29: 757–759. 1939.

# A TECHNIQUE TO COMPARE VIRULENCE OF ISOLATES OF ALTERNARIA SOLANI ON TOMATO LEAFLETS

FREDERICK L. WELLMAN

(Accepted for publication January 2, 1943)

The early blight (*Alternaria solani* (E. & M.) J. & G.) of the tomato (*Lycopersicon esculentum* Mill.) has long caused serious losses. It is recognized that, in general, Alternaria leaf blight has become in recent years probably the most important tomato defoliation disease. Spraying or dusting with Bordeaux mixture or other copper fungicides is the common control measure for the trouble in the field, but the practice is expensive and the application of Bordeaux mixture to tomatoes at times may have an injurious effect on the plants (3, 4, 11). The desirability of horticulturally acceptable tomato varieties that are resistant to Alternaria is, of course, readily appreciated and need not here be discussed. Furthermore, spraying uses strategic materials, both in spray chemicals and in the spraying or dusting equipment, that are required in the present war emergency. All this has given further impetus to increased effort of experiment station workers toward the development of Alternaria-resistant varieties of tomatoes that may replace the present susceptible commercial varieties. The technique herein described was developed as part of a program leading to such an end.

Rands (7), Kunkel (5), Bonde (2), as well as others, have observed that *Alternaria solani* is quite variable in cultural characters. Bonde (2) seems to have been the only worker who has made an extensive study of the differences that may be found in the virulence of isolates of the organism, and his work dealt only with tests on potato.

The technique described by the writer is dependent upon the standardization and control of as many factors as possible. The most favorable conditions for the growth of Alternaria and for the leaf-spot attack are known (6, 7, 8), and these were corroborated before being incorporated into the experimental procedure.

## MATERIALS

A single commercial strain of Marglobe tomato was used as the standard host for these studies. It was found that the reactions to inoculum on leaflets of this strain were very consistent. Other strains of Marglobe, however, as well as other varieties, such as Bonny Best, Globe, and Pritchard, were also about equally susceptible to attack.

A number of tests of isolates of *Alternaria solani* were made to compare the severity of disease produced by 7 of the most widely differing isolates that had been collected from typical, naturally occurring Alternaria spots on tomato plants from Maryland fields. The specific identity of these cultures was further established by examinations of both spore and cultural characters by Charles Drechsler and me, and by proof of pathogenicity.

Two culture media were used. First, an agar medium I devised according to the following formula: agar 12 g., water-ground white corn (maize) meal 12 g., cane sugar 12 g., tap water 1000 cc., autoclaved at 15 pounds' pressure for 20 minutes, and used without straining. Second, the Tochinai liquid medium (9, 1).

The incubation chambers in which the growing inoculated plants were held were of the type commonly used in such phytopathological studies. They were mounted on a bench in a warm greenhouse, had slanting glass tops shaded with cheesecloth, and a layer of wet sphagnum moss in the bottom. The air temperature inside the chambers ranged from 23 to 27° C.

### PREPARATION OF CULTURES FOR INOCULUM

The best method of growing Alternaria isolates for inoculation purposes were studied. After tests with both liquid and agar media it was found that the most satisfactory results were from inoculum grown on agar. A piece of young fungus mat about 15 mm. in diameter was planted in the middle of an agar plate. After the colony had grown to about 50 mm. in diameter, 6-mm. disks were cut from just inside its edges as standard bits for inoculum.

It was found practically impossible at times to handle some cultures in certain series so that they would be available to compare with other types of the organism that grew more slowly in cultures. Experiments showed, however, that when colonies had grown to the best size for use as inoculum, the Petri-dish cultures could be refrigerated (6 to 8° C.) in a moist chamber and kept for at least two weeks, after which they could be used for inoculum without any appreciable change in relative virulence of the organism.

### METHODS OF INOCULATION AND INCUBATION OF PLANTS FOR VIRULENCE TESTS

Determination of the comparative virulence of isolates of an organism is manifestly dependent on the results of tests on inoculated host plants. Many different methods were used in making these tests. Leaflets were inoculated on growing plants, and excised leaves and leaflets also were inoculated. Both attached and excised leaflets were then incubated in moist chambers, in light and in darkness, and under a variety of other controlled environments. While in certain instances tests with excised parts were promising, there also were unexplained failures. Often soft-rot bacteria invaded excised leaflets and inhibited and disturbed Alternaria-infection results. It also was believed that pathogenicity or virulence determinations on an excised host organ might perhaps be less conclusive than kindred observations on the same organs attached to growing plants.

The plants most successfully employed in these studies were grown in a warm greenhouse and were ready for use when they had reached the 5-leaf stage, which was usually about a month after sowing. Seedlings were transplanted into 3-inch pots at the time true leaves appeared as a small bud between the well developed cotyledons. The soil used was moderately fertile greenhouse compost without additional enrichment.

Plants were selected for inoculation that had two full-grown primary or "smooth" leaves at the base above the cotyledons. The first adult or "rough" leaf above the primaries was considered as No. 3. Its nearly expanded terminal leaflets were chosen for inoculation. In preliminary work it was found that results on primary leaflets were irregular, and that lesions on leaf No. 4 tended to be smaller than those on leaf No. 3.

In all tests, plants were selected for uniformity from a large population and arranged in rows in the inoculation chamber. They were oriented so that the tip of leaf No. 3 was pointing in one direction for ease of access by the operator, and rows were so spaced that no inoculated leaflets touched each other or the top or sides of the chamber. The plants were thoroughly watered before inoculating, particular attention being given to gentle washing of the leaflets that were to be used for test purposes. These leaflets were atomized both before and after inoculation.

The technique employed by Andrus *et al.* (1) in their work on Alternaria leafspot resistance in tomato was investigated. This method satisfactorily demonstrated relatively wide differences in virulence between isolates; but, when finer distinctions were sought, the results were too irregular and likely to be confused. Observations at the Bureau of Plant Industry Station, Beltsville, Maryland, indicated that unavoidable mechanical injuries from top-dipping and the variations in quantity and placement of inoculum were the main sources of these irregular results.

Plants grown in pots were watered and arranged in the incubation chamber as described above. A small spatula made from a piece of stiff platinum wire (B. & S. gauge 16) was used to transfer disks of inoculum from Petri-dish cultures onto the plants. If the leaflets were dry, plants were atomized with water lightly just before inoculation and a disk was placed fungus side up on the upper side and at the center of each leaflet. Plants were invariably atomized again lightly at the time the chambers were closed. Inoculated leaflets were examined each day for disease progress.

In judging the right time to record results, a standard culture of known virulence was always used for reference purposes. Data were taken when a majority of leaflets inoculated with this culture had reached the most severe stage of injury; that is, when leaflets were fully involved but remained attached to the main petiole, when necrosis of petiolules had occurred with severe epinasty of the whole conpound leaf, and when about a third of the infected leaves had dropped because of disease effects. The incubation period varied from 4 to 7 days, but the most clear-cut data were generally secured at the end of 5 days.

Tests on plants handled as described are fairly economical of space and time. In one chamber 2 ft. 9 in. wide by 15 ft. long, 200 plants were easily accommodated for inoculation purposes. A very satisfactory test unit consisted of 10 plants on each of which 3 leaflets were inoculated, thus giving 30 numerical estimates of virulence for each test. It is believed that 5 plants per test would be satisfactory in some experiments. It took two

workers about 4 hours to arrange and inoculate 380 plants (1140 leaflets inoculated) in the incubation chambers, and less than two hours to estimate and record relative virulence observed on these plants.

### PROGRESS OF SYMPTOMS OF ALTERNARIA LEAF ATTACK

It was imperative to know the changes from day to day in appearance of a typical alternaria-infected leaflet. These changes were recorded as the basis for the numerical evaluation of relative virulence. The progress in symptoms of Alternaria leaf spot was studied under both field and laboratory conditions. Notes of disease progress on inoculated plants, were made 3 times daily in the greenhouse, while observations in the field were made once a day. In the greenhouse, rapidity of attack differed and it would seem was correlated with the relative virulence of the isolates used for inoculum. However, the symptoms always followed a consistent course.

TABLE 1.—*Typical observed progress of macroscopic symptoms of Alternaria solani attack of an inoculated leaflet on a Marglobe tomato plant, under controlled experimental*[a] *conditions*

| Time observed | Symptom appearance (macroscopic) | | Epinasty effects | Virulence value[b] |
|---|---|---|---|---|
| | Of inoculum | Of invaded area | | |
| Day 0, afternoon | Inoculum applied | No infection | None | 0 |
| " 1, morning | " powdery | ..... | ..... | 0 |
| " 1, noon | " pubescent | ..... | ..... | 0 |
| " 1, afternoon | Hyphae penetrating leaf | Faint stippling | Questionable tilt of leaflet | 1 |
| " 2, morning | " | Irregular narrow ring | Petiolule deflected | 3 |
| " 2, noon | Aerial hyphae collapsed | Wider ring | Slight deflection of whole leaf | 3 |
| " 2, afternoon | " | Zone broader | Whole leaf pushed downward | 4 |
| " 3, morning | " | Infected spot 18 mm. diameter | " | 6 |
| " 3, noon | " | Infected spot 20 mm. diameter | " | 7 |
| " 3, afternoon | " | Spot same size; necrotic fleck along vein | " | 8 |
| " 4, morning | " | Spot same size; necrotic fleck along vein; leaflet tip collapsing | " | 10 |
| " 4, noon | Hyphae growing on surface | Spot same size; several necrotic flecks along vein; collapse extending to base | " | 12 |
| " 4, afternoon | " | Spot same size; necrotic flecks along vein; whole leaflet necrotic | " | 13 |
| " 5, morning | Aerial hyphae very abundant | Leaflet shriveled | Abscission | (14) |

[a] See accompanying text of this paper for methods employed in this study and controlled environment involved.

[b] For these virulence values, see diagrams in figure 1 and the description of those diagrams in the text.

Table 1 is a record of successive appearance of the symptoms of one typical spot as observed on one leaflet in a single experiment. This succession of appearances has been found in general to occur in the field, although the spots started as much smaller lesions, were slower in development, and were often arrested at a comparatively early stage on the mature leaves under the less sheltered, drier, outside conditions.

It will be seen in table 1, that first macroscopic symptoms of infection in the greenhouse appeared at about the close of 24 hours of incubation. In an equal period following, there was slow extension of a border of tissue darkened by fungus attack around the inoculum-disk. During the overnight period that followed the first 48 hours of incubation, the most rapid spread of the directly invaded, darkened leaf area was observed. Following this, small, irregular, necrotic areas were soon found along and between the veins in the more remote portions of the leaflet, even back into the petiolule, and sometimes affecting adjacent inoculated leaflets. Microscopic examination and isolation studies disclosed no fungal growth in these spots appearing at some distance away from the main leaf lesion. Increasing general flaccidity of the lamina followed appearance of these latter systemic effects; and this ended in shriveling of the leaves bearing the severely diseased leaflets.

In my work on fusarium wilt of tomato (10) epinasty appeared among the earliest symptoms. Infection of tomato leaflets by Alternaria also causes an epinasty, apparent very soon after the first symptoms of leaf attack are macroscopically visible. Under the described greenhouse conditions, epinastic effects increase in severity along with increase in the foliar lesion and end in dropping (abscission) of the whole compound leaf if the terminal leaflets are very severely affected. Leaflets by themselves are not dropped.

### NUMERICAL EVALUATION OF DISEASE SEVERITY ON INOCULATED LEAFLETS

The usual progress of symptoms under a standard set of conditions was determined from many studies. On the basis of this progression, comparisons were made of the amount of disease caused by isolates that differed in various ways, and a numerical grading of virulence was then devised. The grades were readily recognizable, and these grades or values are diagrammatically represented in figure 1 (see also table 1 for comparison of disease progress with numerical values).

An inoculated leaflet showing no infection was given the value 0. The first sign of infection was stippling about the inoculation disk, valued at 1 or 2, depending upon severity. When a discrete, moderately narrow collar of infecting mycelium developed on a narrow band of darkened tissue around the inoculation disk, the infection was valued at 3. Infection from 1 to 3, inclusive, was considered "mild." As the infection spread beyond the delimited collar, the discolored area increased and the different comparative widths were given the "medium" values of 4, 5, 6, and 7. At 7 there

appeared to be an approximate maximum point beyond which little if any further expansion of the dark colored infected area occurred around the inoculum. The more virulent isolates caused symptoms that appeared as small to large isolated water-soaked areas in the leaflet tissue, often some distance from the seat of infection. When these occurred the "medium severe" values 8, 9, and 10 were applied, depending upon intensity of effect. As infection increased to "severe," the water-soaked areas enlarged and the

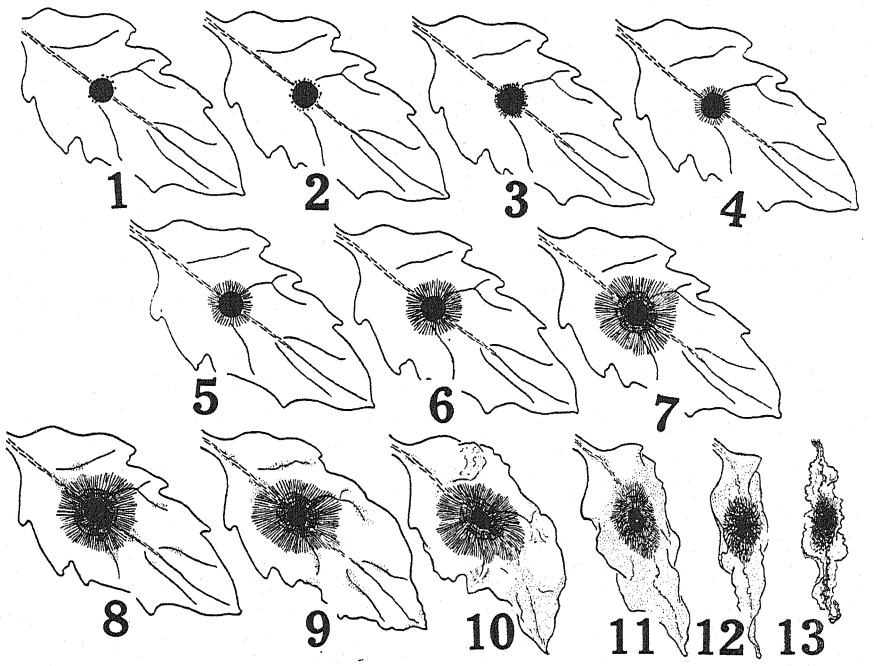



FIG. 1. Diagrammatic representation of progressive stages in macroscopic symptoms of attack on tomato leaflets after inoculation, using a disk cut from a culture of *Alternaria solani*. The disk is represented by black spot at center of leaflet. Virulence values were based on 14 stages of attack as follows: No infection (not illustrated) = 0; few small spots around inoculum = 1; ring of small infection spots around inoculum = 2; spots coalesced into narrow, irregular dark halo = 3; width of halo about one-third diameter of inoculum = 4; halo width slightly less than diameter of inoculum = 5; halo about same as inoculum diameter = 6; halo somewhat wider than diameter of inoculum = 7; few small water-soaked flecks outside halo = 8; somewhat more advanced than 8, small tip portion of leaflet flaccid = 9; coalescence of water-soaked spots, half of leaflet wilting = 10; water-soaking and wilting of about three-fourths of leaflet = 11; all leaflet wilted except small areas at base = 12; leaflet entirely wilted and shriveled = 13; like 13 but whole compound leaf dropped (not illustrated) = 14.

leaflets became flaccid at the tip. Finally, the whole leaflet, except the base near the petiolule was involved, and hung limply. These reactions were valued at 11 and 12. When a limp leaflet was found shriveled and crinkled around the edges it was valued at 13. In the "very severe" infections, the whole compound leaf to which 3 diseased terminal leaflets were attached would drop, and this being an extreme severity, it has been suggested that it might be given a value of 14.

TABLE 2.—*Results of successive tests*[a] *on Marglobe tomato plants inoculated with isolates of Alternaria solani to demonstrate comparative virulence and small difference of reaction between isolates*

| Test number and date started | Index of virulence obtained from isolate[b] indicated: | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G |
| | *Mean* | *Mean* | *Mean* | *Mean* | *Mean* | *Mean* | *Mean* |
| 1 May 11 | 10.1 ± 0.15 | 9.5 ± 0.18 | 4.3 ± 0.20 | 7.4 ± 0.20 | ..... | 1.7 ± 0.22 | ..... |
| 2 May 25 | 10.0 ± 0.21 | 10.2 ± 0.01 | 4.0 ± 0.32 | ..... | ..... | ..... | ..... |
| 3 June 8 | 10.1 ± 0.20 | 18.8 ± 0.39 | 3.7 ± 0.18 | ..... | 4.4 ± 0.25 | 1.4 ± 0.09 | ..... |
| 4 June 22 | 12.3 ± 0.19 | 12.1 ± 0.26 | 3.9 ± 0.26 | ..... | ..... | ..... | ..... |
| 5 July 13 | 9.0 ± 0.01 | 11.0 ± 0.32 | 4.7 ± 0.25 | ..... | ..... | ..... | 1.6 ± 0.02 |
| 6 August 10 | 11.8 ± 0.01 | 9.8 ± 0.24 | 2.8 ± 0.02 | 6.8 ± 0.24 | 4.9 ± 0.02 | 1.5 ± 0.10 | ..... |
| 7 August 24 | 9.0 ± 0.26 | 11.1 ± 0.26 | 3.3 ± 0.02 | 6.3 ± 0.27 | 4.6 ± 0.22 | 1.6 ± 0.11 | 1.7 ± 0.02 |
| Mean | 10.8 ± 0.11 | 10.6 ± 0.11 | 3.8 ± 0.09 | 6.8 ± 0.16 | 4.6 ± 0.13 | 1.5 ± 0.10 | 1.7 ± 0.01 |

[a] For technique involved see description given above in this paper. Readings were recorded from 30 leaflets for each test, from which the mean (index of virulence) with its standard error were obtained. For diagrams representing approximate virulence values see figure 1. Compare also with table 1.

[b] Isolates selected after preliminary trials of relative disease-producing capacities and found to be different in degrees of virulence.

### CONSISTENCY OF RESULTS IN REPEATED TESTS

In developing the technique incident to the study of relative differences in virulence of Alternaria isolates on tomato leaflets, it is essential that a test of this kind be regularly reproducible, and that virulence readings be consistent in relative magnitude on successive trials. Consecutive tests were run (Table 2), using the 7 isolates as sources of inoculum and following the experimental technique already described. These isolates were selected on the basis of their proved wide range of difference in severity of disease effects.

Each isolate was recultured at least twice during the series of tests. A new series of plants and cultures was grown for each test. Three of the isolates were relatively stable, grew well in culture, and were tested 7 times during 4 months. Fewer tests were made with the others because of slower growth or the need for more repeated reculturing due to notably wide variations in cultural characters.

From table 2 it is evident that standard errors were small, results were reasonably consistent from one test to another, and dependable comparisons could be made of virulence indices. The largest differences between isolates are obvious: A and B, having mean indices of about 10.5 contrasted with F and G having mean indices at about 1.5. Smaller differences are also demonstrable: C having a mean index of $3.8 \pm .09$ compared with D having an index of $6.8 \pm .16$; or E having an index of $4.6 \pm .13$ compared with F having an index of $1.5 \pm .10$. Close comparisons should be attempted between single evaluations of isolates only when they run within a single test. Similarly, limited numbers of repeated evaluations should be compared only when involved in completely comparable sets or tests.

### SUMMARY

A laboratory technique was devised to test the virulence of isolates from leaf lesions of tomato early blight (*Alternaria solani*).

Tomato seedlings, about a month old, were grown under standard conditions, and leaflets attached to plants were selected for testing virulence of isolates.

Cultures of the pathogen were grown on agar under standard conditions and standardized pieces of the cultures were used for inoculum.

The course of symptoms of attack was studied under controlled conditions of incubation and compared with field observations.

Progressive symptoms on diseased leaflets were classified according to numerical grades, and these numbers were used as virulence values.

Controlled inoculation studies were made and data were compared statistically. Reactions were found to be reasonably consistent from one test to the next, and the relative virulence was expressed on a numerical basis.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND.

LITERATURE CITED

1. ANDRUS, C. F., G. B. REYNARD, and B. L. WADE. Relative resistance of tomato varieties, selections, and crosses to defoliation by Alternaria and Stemphylium. U. S. Dept. of Agr. Circ. 652. 1942.
2. BONDE, R. Physiological strains of *Alternaria solani*. Phytopath. 19: 533–548. 1929.
3. DOOLITTLE, S. P. Tomato diseases. U. S. Dept. of Agr. Farmers' Bull. 1934. 1943.
4. EDGERTON, C. W., and C. C. MORELAND. Diseases of the tomato in Louisiana. Louisiana Agr. Exp. Stat. Bull. 142, 1913.
5. KUNKEL, L. O. A method of obtaining abundant sporulation in cultures of *Macrosporium solani* E. and M. Mem. Brooklyn Bot. Gard. 1: 306–312. 1918.
6. MOORE, W. D. Some factors affecting the infection of tomato seedlings by *Alternaria solani*. Phytopath. 32: 399–403. 1942.
7. RANDS, R. D. Early blight of potato and related plants. Wisconsin Agr. Exp. Stat. Res. Bull. 42. 1917.
8. WEBER, G. F., and G. B. RAMSEY. Tomato diseases in Florida. Florida Agr. Exp. Stat. Bull. 185. 1926.
9. WELLMAN, F. L. A technique for studying host resistance and pathogenicity in tomato Fusarium wilt. Phytopath. 29: 945–956. 1939.
10. ————. Epinasty of tomato, one of the earliest symptoms of Fusarium wilt. Phytopath. 31: 281–283. 1941.
11. WILSON, J. D., and H. A. RUNNELS. The effect of various spray materials on tomato transplants. Ohio Agr. Exp. Stat. Bimo. Bull. 22: 58–65. 1937.

# USTILAGO STRIAEFORMIS. I. GERMINATION OF CHLAMYDOSPORES AND CULTURE OF FORMA AGROSTIDIS ON ARTIFICIAL MEDIA[1]

K. W. KREITLOW

(Accepted for publication November 13, 1942)

The widespread occurrence of *Ustilago striaeformis* (Westd.) Niessl. on different species of grasses has led several authors to investigate the life history of the organism. An interesting feature of these investigations is the discrepant reports regarding the germinability of fresh smut chlamydospores and the difficulty of securing cultures of the organism on artificial media. Davis (1, 2, 3, 4), made extensive collections of *U. striaeformis* on *Agrostis alba* L., *Phleum pratense* L., *Poa pratensis* L., *and Poa annua* L. None of the collections of fresh chlamydospores that he tested germinated more than a small percentage. As a result, Davis investigated factors that influenced germination of the smut chlamydospores. From the work so conducted, he concluded that an after-ripening period was prerequisite for successful germination. Despite the fact that he tried many times, Davis failed to secure cultures from after-ripened chlamydospores.

Fischer (5), working with a race of *Ustilago striaeformis* that occurred on *Agropyron pauciflorum* (Schw.) Hitchc.[2] and *Elymus glaucus* Buckl. experienced no difficulty in germinating fresh chlamydospores of the organism. The race with which Fischer worked grew readily on artificial media and produced numerous sporidia. The cultural studies conducted by Fischer represented the first successful cultivation of *U. striaeformis* on artificial media.

During the past year, germination tests were conducted with chlamydospores from smutted plants of *Agrostis alba*. Most collections of diseased plants failed to yield germinable chlamydospores; however, one of the collections provided spores that germinated 50–75 per cent. Since an abundant source of germinable chlamydospores was supplied by this collection, it was used for most of the studies embodied in this report.

## MATERIALS AND METHODS

During the fall of 1941, 49 smutted plants of *Agrostis alba* were transplanted to a greenhouse for observation. The diseased plants were removed from 49 sod plugs obtained from 12 widely separated pastures located in the central part of Pennsylvania. After each plant was established in a 4-inch pot, fresh chlamydospores from sori in the leaves were tested for germinability.

[1] Contribution No. 43, of the U. S. Regional Pasture Research Laboratory, Bureau of Plant Industry, Agricultural Research Administration, U. S. Department of Agriculture, in cooperation with the northeastern states.

[2] Now known as *A. trachycaulum* (Link) Malte.

All germination tests were conducted in quadruplicate on microscope slides that contained paraffin wells. The wells were prepared by dipping an 18 mm. glass ring into melted paraffin. The glass ring was then touched to a slide and a paraffin ring left superimposed. The process was repeated so that four paraffin wells were deposited on each microscope slide. By placing the wells along one side of the slide, sufficient space was left below each well for labeling.

### RESULTS OF GERMINATION TESTS

When fresh chlamydospores from each of the 49 plants were tested for germination, two plants from one pasture yielded chlamydospores that germinated 50–75 per cent. The plants of this collection, designated 14A, were obtained from a pasture near Lamar, Pennsylvania, on October 24, 1941. Tests of 11 smutted plants subsequently secured from different parts of this same pasture revealed that fresh chlamydospores from 7 of the plants germinated 1–75 per cent, while fresh chlamydospores from the remaining 4 plants failed to germinate. None of the diseased plants collected in other pastures provided more than an occasional germinable chlamydospore.

### EFFECT OF DISTILLED AND TAP WATER ON GERMINATION OF CHLAMYDOSPORES

Since collection 14A provided an excellent source of germinable chlamydospores, tests were conducted to determine what influence different kinds of water had on germination of spores. This was done to determine, if possible, the reason why most chlamydospores of *U. striaeformis* forma *agrostidis* W. H. Davis fail to germinate in a fresh state.

For each test, 3 kinds of water were used; tap water, water distilled in a Pyrex glass still and water distilled in a tin still. A drop of water was placed within each paraffin well and chlamydospores were scraped from sori on diseased leaves and dispersed in the individual drops. Each slide so prepared was then placed in a Petri dish moist chamber and incubated at room temperature for 16–24 hours.

Excellent germination occurred in each of the three kinds of water. This eliminated the possibility that water used for germination tests contained substances likely to inhibit germination of the smut spores.

When germinable chlamydospores were placed in drops of water and each drop was covered with a coverglass, germination failed to occur, suggesting that the spores required free access to atmospheric conditions despite the fact that many spores germinate below the surface of uncovered drops of water.

### INFLUENCE OF ALCOHOL AND ETHER ON GERMINATION OF CHLAMYDOSPORES

Davis (1) discovered that the after-ripening period of smut chlamydospores could be decreased appreciably by treating the spores with chloroform and citric acid. This suggested the possibility that non-germinable

spores of *Ustilago striaeformis* might be treated with different agents and induced to germinate without first subjecting them to an after-ripening period. Preliminary tests were therefore conducted to determine what effect alcohol and ether had on germinable chlamydospores of *U. striaeformis*.

Chlamydospores from collection 14A were treated with 95 per cent ethyl alcohol. Microscopic examination of spores so treated revealed that the spores collapsed almost instantaneously. When the treated spores were placed in water, they regained their turgid condition and appeared uninjured. Germination tests revealed that momentary treatment with 95 per cent ethyl alcohol failed to injure the spores. Longer treatments of 15–30 seconds duration caused complete inhibition of germination despite the fact that the collapsed spores returned to a turgid state.

When germinable chlamydospores of *Ustilago striaeformis* were placed in di-ethyl ether, they survived treatments of several minutes duration despite the fact that the spores collapsed. Germination was reduced considerably by the longer treatments but a short exposure to ether failed to decrease subsequent germination.

Chlamydospores that failed to germinate in a fresh state were then procured from a similar series of *Agrostis alba* plants and treated with ethyl alcohol and ether. All attempts to stimulate germination by treatment with these agents failed.

## EFFECT OF AGE AND POSITION OF SMUT SORI IN THE LEAF ON SPORE GERMINATION

Davis (1) concluded that germination of fresh smut spores from different grasses was not dependent on age of the leaf. He found, however, that the after-ripening period was shorter for spores removed from dead leaves than for spores removed from green leaves. Davis also demonstrated that spores from different sori within a leaf varied with respect to germinability and length of after-ripening period. With this in mind, tests were conducted to determine whether or not similar conclusions could be drawn from the material at hand.

Smut spores from leaves of different plants of collection 14A were tested for germinability. The minimum germination encountered was 1 per cent while the maximum was 75 per cent. A wide range in germination was also discovered when chlamydospores from different plants within a clone were tested for germinability. Variation in germination to the same extent was encountered when similar tests were conducted on different smutted leaves from a single plant. In keeping with Davis' contention, age of leaf was found to be of minor importance in germination of the smut chlamydospores.

Spores were next removed from numerous sori along the entire blade of a leaf and tested for germination. The results confirmed those of Davis who found that spores from different sori in the same leaf varied in ability to germinate. Further tests revealed that mature spores removed from ruptured or unruptured sori or even from different parts of the same sorus differed markedly in ability to germinate.

### INFLUENCE OF STORAGE TEMPERATURE ON SURVIVAL OF CHLAMYDOSPORES

Leaves from smutted plants of collection 14A were stored dry at room temperature, 10° C., and 5° C. to determine what effect storage temperature had on germinability of chlamydospores. Spores in leaves stored 4 days at room temperature failed to germinate. Smutted leaves stored at 10° C. for 30 days yielded germinable chlamydospores, however, germination was reduced from an original 75 per cent to less than 10 per cent. Chlamydospores from leaves stored at 5° C. survived 28 days with a similar reduction in germinability. These tests demonstrated that fresh, germinable spores of *U. striaeformis* were capable of surviving at least 30 days when stored at favorable temperatures.

### CULTIVATION OF USTILAGO STRIAEFORMIS FORMA AGROSTIDIS ON ARTIFICIAL MEDIA

Since excellent germination of fresh chlamydospores occurred in drops of water, the possibility of securing germination on agar media was investigated. For this purpose, drops of 3 per cent potato-dextrose agar were placed aseptically on sterile coverslips. Spores from smutted leaves were next transferred as aseptically as possible to the agar drops, and the coverslips were inverted on glass rings in Petri dish moist chambers.

After 24–36 hours incubation at room temperature, the agar drops inoculated with chlamydospores were examined for spore germination. Each germinated spore usually displayed a single branched or unbranched promycelium 20 to 50 μ long. No sporidia were observed although lateral branches resembling sporidia were frequently noted on rapidly growing promycelia.

Single germinated chlamydospores were then removed from the agar drops by means of a micromanipulator and transferred to fresh drops of agar. About 25 per cent of the chlamydospores so handled continued their growth and developed into strictly mycelial colonies. A typical germinated spore with lateral mycelial branches is shown in figure 1, A. The mycelial type of growth secured in cultures of *U. striaeformis* forma *agrostidis* is a marked contrast to the sporidial type of growth obtained by Fischer (5) from *U. striaeformis* forma *hordei* G. W. Fischer.

Of 10 single-chlamydospore cultures secured from diseased plants of *Agrostis alba*, two different biotypes were observed. Colonies of one form were characterized by a flat, lustrous, ridged growth that was a light buff[3] on potato-dextrose agar. This colony-form is illustrated in figure 1, B. Colonies of the other form were slower growing and were of a fluffy, raised mycelial type of growth that was also light buff. A representative of this colony-form is shown in figure 1, C.

The effect of different temperatures on growth of the cultures was determined by growing the organism in 250-cc. flasks that contained 50-cc. aliquots of potato-dextrose agar. Each culture was grown at 7 different

[3] Ridgway, R. Color standards and color nomenclature. 43 pp., 53 colored plates. (Washington, D. C.). 1912.

temperatures arranged at 5° intervals from 5° C. to 35° C. Best growth occurred at 20° C. and 25° C. No growth occurred at 35° C. and only feeble growth at 5° C.

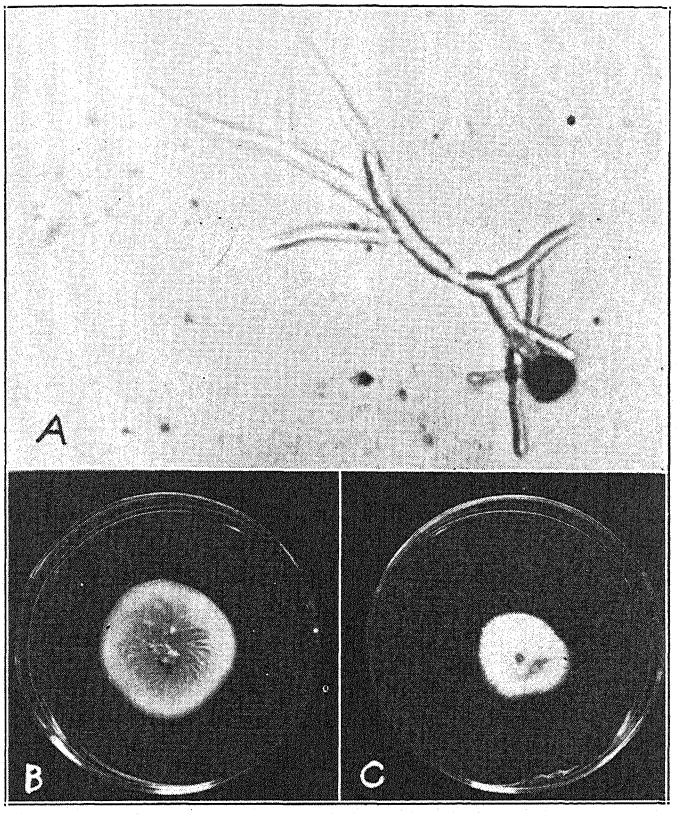



FIG. 1. Germinating chlamydospore and cultures of *Ustilago striaeformis* forma *agrostidis*. A. Chlamydospore after 72 hours on potato-dextrose agar. ×636. B and C. Cultures on potato-dextrose agar in 90-mm. Petri dishes. Growth after 19 days at 27° C.

## DISCUSSION

The discovery that an occasional collection of *Ustilago striaeformis* forma *agrostidis* is capable of germinating immediately when removed from the plant gives added incentive to the investigation of why most collections of this smut fail to germinate unless they are subjected to an after-ripening period. It seems probable that the collection that germinated readily, in contrast to the majority of collections that fail to germinate, is a physiologic form or strain within the race *U. striaeformis* forma *agrostidis*, although the data available at present do not eliminate the possibility of a differential effect of the host plant upon this character. The germinable strain may be further subdivided into biotypes based on cultural characteristics as indicated in this paper.

The question then arises whether or not *Ustilago striaeformis* is in reality a collection of species and should be reclassified with respect to natural hosts and inoculation studies on related and unrelated hosts. Work by Davis, although not conclusive showed that chlamydospores of *U. striaeformis* taken from timothy failed to infect redtop when cross-inoculation tests were carried out with these and other grasses. On the basis of inoculation tests, spore size, and spore morphology, Davis concluded that distinct biological races of the smut occurred on *Agrostis alba, Phleum pratense, Poa pratensis* and *Poa annua.* Fischer's extensive inoculation tests (5), demonstrated that the race of smut with which he worked readily infected species of *Agropyron, Elymus,* and *Hordeum* but failed to infect species of *Poa, Phleum, Agrostis,* etc. Only the weight of further experimental evidence will demonstrate whether or not *U. striaeformis* is an assemblage of species or forms.

## SUMMARY

A collection of *Ustilago striaeformis* forma *agrostidis,* the chlamydospores of which germinate without an after-ripening period, is described.

Chlamydospores selected from the same sorus or from different sori on the same plant vary in ability to germinate.

Single-chlamydospore cultures of this race of the organism were grown on artificial media.

Growth of the cultures was strictly mycelial in form and could be differentiated into 2 biotypes.

## LITERATURE CITED

1. DAVIS, W. H. Spore germination of *Ustilago striaeformis.* Phytopath. 14: 251–267. 1924.
2. ————. Life history of *Ustilago striaeformis* (Westd.) Niessl. which causes a leaf smut in timothy. Jour. Agr. Res. [U. S.] 32: 69–76. 1926.
3. ————. Two physiologic forms of *Ustilago striaeformis.* Phytopath. 20: 65–74. 1930.
4. ————. Summary of investigations with *Ustilago striaeformis* parasitizing some common grasses. Phytopath. 25: 810–817. 1935.
5. FISCHER, GEORGE W. Fundamental studies of the stripe smut of grasses (*Ustilago striaeformis*) in the Pacific Northwest. Phytopath. 30: 93–118. 1940.

# DISAPPEARANCE OF VIRUS FROM MOSAIC-DISEASED SUGARCANE PLANTS

I. L. Forbes and P. J. Mills

(Accepted for publication October 26, 1942)

Sugarcane is propagated vegetatively by planting stalks. In varieties very susceptible to mosaic, all plants developing from a diseased seed piece usually show mosaic. In some varieties, however, one or more buds on a diseased seed piece may develop into plants free from mosaic symptoms. This observation has been reported by several workers. Lyon (3), in Hawaii, reported that shoots of the Lahaina cane threw off mosaic during the growing season. Also, healthy plants were observed to develop from stubble pieces of cane that had mosaic the previous season. Kunkel (2) also observed that certain mosaic-diseased stools of sugarcane often recovered. When seed cane was taken from these stools and planted, the young shoots remained healthy for a time, though later some developed mosaic. Kunkel considered this as probably a new infection. East (1), in Cuba, observed much the same thing. He found that some of the plants remained healthy for over a year, while others became infected within 3 months and then recovered again. He set forth two hypotheses that might explain this. The host might kill the virus and throw off the symptoms, remaining partially resistant for a time, or it might reduce the virulence of the virus for a time, in which case symptoms might again appear if the resistance of the host was lowered. Tims and Edgerton (4) in Louisiana reported that the relative resistance of P.O.J. 213 and P.O.J. 228 was to a considerable extent due to the ability of the plants to throw off the disease and to produce virus-free buds. Infected stalks of these varieties, when planted produced both healthy and diseased shoots. The resistant varieties produced a much greater number of healthy shoots than did the susceptible varieties. They reported also that mosaic symptoms commonly disappeared on the resistant P.O.J. canes. Many plants showing the disease in early summer were apparently healthy in the fall. In a later investigation, Tims, Mills, and Edgerton (5) found recovery from mosaic to be common in C.P. 29/320 and C.P. 28/11, and occasional in C.P. 28/19. The infective virus was not always absent from plants which had apparently recently recovered from the disease, since in some cases the disease was produced by inoculating juice from such plants into healthy plants. The percentage of infection, however, was not high.

In Louisiana it has frequently been observed that when mosaic-diseased stalks of sugarcane are planted some buds on such seed pieces develop into plants free from mosaic symptoms. It also has been determined with certain varieties, that mosaic symptoms disappear very commonly in the field from some plants during the growing season. The question naturally arises,—

are such plants free from the mosaic virus, and are they immune from infection by the same virus?

In order to obtain more definite information in regard to the virus condition of sugarcane plants in which the mosaic symptoms had disappeared, it seemed desirable to carry out further tests. The results of experiments to determine whether or not plants without mosaic symptoms, grown from diseased stalks, or plants from which symptoms disappeared during the growing season still contained the mosaic virus, and whether or not such plants were immune from reinfection with the same virus are reported in this paper. The results were largely obtained from inoculation tests.

In all the infection tests the mosaic was transmitted by juice inoculations. Diseased shoots were macerated in a food chopper and the juice extracted from the material by pressure in cheesecloth. Several cc. of juice was placed in the whorl of leaves at the top of the plant and inoculation was effected by pricking through the spindle of the plant with fine needles that carried the virus into the young, growing tissues of the leaves. Under good growing conditions, mosaic symptoms usually showed in the newly developed leaf tissues in two to three weeks. For some unknown reason the percentage of inoculated plants that developed mosaic was often rather low, especially with varieties in which the rate of pick-up of mosaic in the field was slow. As the work was carried on in fields well isolated from mosaic-diseased cane, very seldom were mosaic symptoms observed in plants not inoculated. The period of time during which infection could be obtained by artificial inoculation was limited to the months of May and June. It was practically impossible to obtain satisfactory infection after early July. Whether or not this was due to high temperatures during this portion of the growing season, to high light intensities which might possibly inactivate the virus *in vitro* during the time required for inoculation, or to some other factor is not known at the present time. Due to this fact, however, it was impossible to determine by inoculation whether or not plants from which mosaic symptoms disappeared during the current season were susceptible to reinfection by the mosaic virus.

### SUSCEPTIBILITY OF RECOVERED PLANTS

In order to determine whether or not mosaic-free plants grown from diseased stalks were immune from infection, the following experiments were made.

In a test started March 16, 1942, in the greenhouse, 10 symptomless plants of C.P. 28/19, grown from diseased stalks, were inoculated with a virus from C.P. 28/19. As checks, 10 healthy plants of the same variety and 10 healthy plants of the variety Co. 281, grown from healthy stalks, were inoculated with the same virus. Final results were read May 6. Of the 10 symptomless plants inoculated, 6 developed mosaic and 4 no mosaic. Of the 10 check plants of C.P. 28/19 inoculated, all developed mosaic. Of the 10 check plants of Co. 281 inoculated, 8 developed mosaic and 2 no mosaic.

On May 11, 1942, an experiment was made in the field in which 7 symp-

tomless plants of C.P. 28/19 grown from mosaic-diseased stalks were inoculated with C.P. 28/19 virus; and 5 symptomless plants of C.P. 33/243, grown from diseased stalks, were inoculated with C.P. 33/243 virus. As checks, 5 plants of C.P. 28/19 and 5 plants of C.P. 33/243, grown from healthy stalks, were inoculated with mosaic virus from C.P. 28/19 and C.P. 33/243. Final results were read June 25. The 7 symptomless plants of C.P. 28/19 developed mosaic. Of the 5 symptomless plants of C.P. 33/243, 4 developed mosaic and 1 no mosaic. In the checks, 4 of the 5 C.P. 28/19 plants and all 5 of the C.P. 33/243 plants developed mosaic.

From the results obtained it is apparent that sugarcane plants that have recovered from the mosaic disease or symptomless plants that have developed from mosaic-infected seed cane will take the disease when inoculated and are in no sense immune.

## ABSENCE OF VIRUS IN RECOVERED PLANTS

In order to determine whether active infective virus was present in sugarcane stalks which had apparently recovered from the mosaic or in stalks that had developed from mosaic-infected seed pieces, a number of tests were carried on during the seasons of 1940, 1941, and 1942. In the tests, juice was expressed from symptomless plants that had developed from infected seed pieces and from plants from which the mosaic symptoms had disappeared. The results of the tests are included in the table. In all experiments, for controls healthy plants were inoculated with virus from mosaic-infected plants.

In the 17 tests (listed under 11 experiment numbers in table 1) in which 425 plants were inoculated with juice from a total of 59 symptomless plants, only one developed the mosaic disease. This one can be assumed to have developed from natural infection. Of the checks inoculated with virus from mosaic-infected plants, 43.1 per cent developed mosaic. The evidence seems to be conclusive that the symptomless plants did not contain active, infective virus.

## SUMMARY

Experiments were carried out during 1940, 1941, and 1942 involving inoculations of healthy cane plants with juices from (1) mosaic plants, (2) symptomless plants grown from diseased seed pieces, and (3) plants from which mosaic symptoms had disappeared during the current season.

These experiments comprised seventeen sets of comparisons, involved 6 varieties of sugarcane, and included 790 inoculations.

In each of the 17 experiments inoculations with juices from mosaic canes were followed by a development of mosaic symptoms. The percentage of infection ranged from 10 to 80 per cent.

In 16 of the 17 experiments inoculations with juices from symptomless canes grown from diseased seed pieces, and inoculations with juice from plants from which symptoms had disappeared during the current season

TABLE 1.—*Results of inoculations with (1) juice from symptomless plants grown from mosaic-diseased seed cane, (2) with juice from plants from which symptoms had disappeared during the current season, and (3) with virus from mosaic plants (checks)*

| Source of inoculum | | | Experiment number and date initiated | Inoculation results | | | |
|---|---|---|---|---|---|---|---|
| Variety | Number symptom-less plants | Number mosaic plants | | Varieties inoculated | Number plants inoculated | Number plants developing mosaic | Number plants no mosaic |
| C.P. 29/320 | 3 | | I—May 1940 | C.P. 28/70 | 15 | 1[a] | 14 |
| C.P. 29/320 | | 3 | | C.P. 28/70 | 15 | 2 | 13 |
| C.P. 28/19 | 3 | | I—May 1940 | C.P. 28/19 | 15 | 0 | 15 |
| C.P. 28/19 | | 3 | | C.P. 28/19 | 15 | 6 | 9 |
| C.P. 28/19 | 3[b] | | I—May 1940 | Co. 281 | 15 | 0 | 15 |
| C.P. 28/19 | | 3[b] | | Co. 281 | 15 | 5 | 10 |
| C.P. 33/243 | 3 | | I—May 1940 | C.P. 33/243 | 15 | 0 | 15 |
| C.P. 33/243 | | 3 | | C.P. 33/243 | 15 | 8 | 7 |
| C.P. 33/243 | 3[b] | | I—May 1940 | C.P. 28/70 | 15 | 0 | 15 |
| C.P. 33/243 | | 3[b] | | C.P. 28/70 | 15 | 8 | 7 |
| C.P. 29/320 | 4 | | II—June 1940 | C.P. 28/70 | 20 | 0 | 20 |
| C.P. 29/320 | | 3 | | C.P. 28/70 | 15 | 2 | 13 |
| C.P. 33/243 | 4 | | II—June 1940 | Co. 281 | 20 | 0 | 20 |
| C.P. 33/243 | | 3 | | Co. 281 | 15 | 3 | 12 |
| C.P. 29/320 | 5 | | III—May 1941 | Co. 281 | 25 | 0 | 25 |
| C.P. 29/320 | | 5 | | Co. 281 | 25 | 20 | 5 |
| C.P. 29/320 | 5 | | IV—June 1941 | Co. 281 | 25 | 0 | 25 |
| C.P. 29/320 | | 5 | | Co. 281 | 25 | 17 | 8 |
| C.P. 29/320 | 5[b] | | IV—June 1941 | C.P. 28/70 | 25 | 0 | 25 |
| C.P. 29/320 | | 5[b] | | C.P. 28/70 | 25 | 10 | 15 |

TABLE 1.—(*Continued*)

| Source of inoculum | | | Experiment number and date initiated | Inoculation results | | | |
|---|---|---|---|---|---|---|---|
| Variety | Number symptom-less plants | Number mosaic plants | | Varieties inoculated | Number plants inoculated | Number plants developing mosaic | Number plants no mosaic |
| C.P. 29/320 | 5 | | V—June 1941 | Co. 281 | 25 | 0 | 25 |
| C.P. 29/320 | | 5 | | Co. 281 | 25 | 17 | 8 |
| C.P. 29/320[b] | 5[b] | | V—June 1941 | C.P. 28/70 | 25 | 0 | 25 |
| C.P. 29/320 | | 5[b] | | C.P. 28/70 | 25 | 10 | 15 |
| C.P. 33/243[c] | 5[c] | | VI—May 1941 | C.P. 33/243 | 25 | 0 | 25 |
| C.P. 33/243 | | 5 | | C.P. 33/243 | 25 | 17 | 8 |
| C.P. 33/243 | 3 | | VII—June 1941 | C.P. 33/243 | 15 | 0 | 15 |
| C.P. 33/243 | 3 | | | C.P. 28/19 | 15 | 0 | 15 |
| C.P. 33/243 | | 6 | | C.P. 33/243 | 30 | 22 | 8 |
| C.P. 28/19 | 1 | | VIII—May 1941 | C.P. 28/19 | 10 | 0 | 10 |
| C.P. 28/19 | | 1 | | C.P. 28/19 | 10 | 4 | 6 |
| C.P. 28/19 | 5 | | IX—June 1941 | C.P. 28/19 | 50 | 0 | 50 |
| C.P. 28/19 | | 5 | | C.P. 28/19 | 50 | 5 | 45 |
| C.P. 29/320 | 4 | | X—June 1941 | Co. 281 | 40 | 0 | 40 |
| C.P. 29/320 | | 4 | | Co. 281 | 40 | 15 | 25 |
| C.P. 29/320[c] | 6[c] | | XI—June 1942 | C.P. 28/70 | 11 | 0 | 30 |
| C.P. 29/320 | | 5 | | C.P. 28/70 | 11 | 6 | 19 |

[a] Interpreted as natural infection.
[b] Same virus as was used to inoculate other variety in same experiment.
[c] Plants from which symptoms disappeared during the current season.

were not followed by a development of mosaic symptoms. In one experiment symptoms developed on one out of 15 plants inoculated. The development of symptoms under these circumstances was attributed the secondary infection in the field.

The results obtained appeared to establish the fact that no infective virus was present in symptomless plants grown from sugarcane seed stalks originally exhibiting mosaic symptoms, nor in plants from which symptoms disappeared during the current season.

Experiments made in 1942 showed that mosaic-free plants grown from diseased seed pieces were not immune from reinfection by the mosaic virus.

## LITERATURE CITED

1. EAST, E. M. Immunity to sugarcane mosaic acquired by the host. Proc. Nat. Acad. Sci. 17: 331–334. 1931.
2. KUNKEL, L. O. Studies on the mosaic of sugar cane. Hawaiian Sugar Planters' Assoc. Exp. Stat. Bull. (Bot. Ser.) 3: 115–167. 1924.
3. LYON, H. L. Three major cane diseases: mosaic, sereh, and Fiji disease. Hawaiian Sugar Planters' Assoc. Exp. Stat. Bull. (Bot. Ser.) 3: 1–43. 1921.
4. TIMS, E. C., and C. W. EDGERTON. Behavior of mosaic in certain sugarcane varieties in Louisiana. Amer. Jour. Bot. 18: 649–657. 1931.
5. ————, P. J. MILLS, and C. W. EDGERTON. Studies on sugarcane mosaic in Louisiana. La. Agr. Exp. Stat. Bull. 263. 1935.

# MOSAIC SPOTS OF FIG FRUITS[1]

IRA J. CONDIT[2] AND W. T. HORNE[3]

(Accepted for publication November 30, 1942)

In earlier papers on fig mosaic,[4,5] brief mention was made of the occurrence of mosaic spots on fig fruits. The wide prevalence of these spots, both on caprifigs and on edible figs, warrants a more detailed account of the manifestations of this disorder.

## MOSAIC SPOT OF CAPRIFIGS

In a bulletin[6] entitled "Caprifigs and caprification," published some 20 years ago, there appeared an illustration of an unusual spotting of caprifigs, with the following caption: "This peculiar spot or blemish is a characteristic of some of the profichi . . . of the Samson (Markarian No. 1). It is found on all trees of the variety and is evidently distributed by cuttings." These blemishes marred the appearance of the figs and prevented them from developing properly. Examination of specimens failed to show any pathogen present. Recent observations have confirmed the opinion previously expressed,[4,5] that this spotting of caprifigs is a manifestation of fig mosaic.

### Occurrence

The mosaic spot of caprifigs has been observed only on the profichi, which, in the hot interior sections of California, mature early in June. The spot has not been seen on any of the mammoni or summer figs, which follow the profichi, or on the mamme figs, in which the fig insects or blastophagas pass the winter. Nor has any similar spot or blemish been noted on the leaves of these trees, although many of the leaves show mosaic spots and malformations.

*The Samson Caprifig.* The spot was first found on the Samson caprifig, a variety probably introduced from Asia Minor in 1882, by G. P. Rixford, and established on the Stanford Ranch at Vina, California. From this introduction many cuttings were propagated and distributed by various persons, including Herbert Samson of Corning and Henry Markarian of Fresno. The latter gave it the variety name Markarian No. 1. The mosaic spot has been present on a certain percentage of profichi of every Samson tree examined in the fig districts of California. No similar spot has been

[1] Paper No. 485, University of California Citrus Experiment Station, Riverside, California.

[2] Associate Professor of Subtropical Horticulture and Associate Subtropical Horticulturist in the Experiment Station.

[3] Professor of Plant Pathology and Plant Pathologist in the Citrus Experiment Station.

[4] Condit, Ira J., and W. T. Horne. A mosaic of the fig in California. Phytopath. 23: 887–896. 1933.

[5] Condit, Ira J., and W. T. Horne. Further notes on fig mosaic. Phytopath. 31: 561–563. 1941.

[6] Condit, Ira J. Caprifigs and caprification. Calif. Agr. Exp. Stat. Bull. 319: 341–377. 1922.

found on profichi of Roeding No. 3, Stanford, or any of the dozen other varieties of caprifigs commonly grown.

Profichi of the best varieties of caprifigs set and mature practically 100 per cent of the crop. Samson figs that are affected by spotting invariably drop prematurely; some drop when small, others become malformed and then drop, while a few reach almost full size and color, even though the spotting is present. Noninfected figs are dark green in color until almost fully ripe, and are symmetrical in shape without prominent external blemishes.

A census of the profichi borne by a Samson tree at Riverside showed that 37.2 per cent of these fruits were visibly spotted.

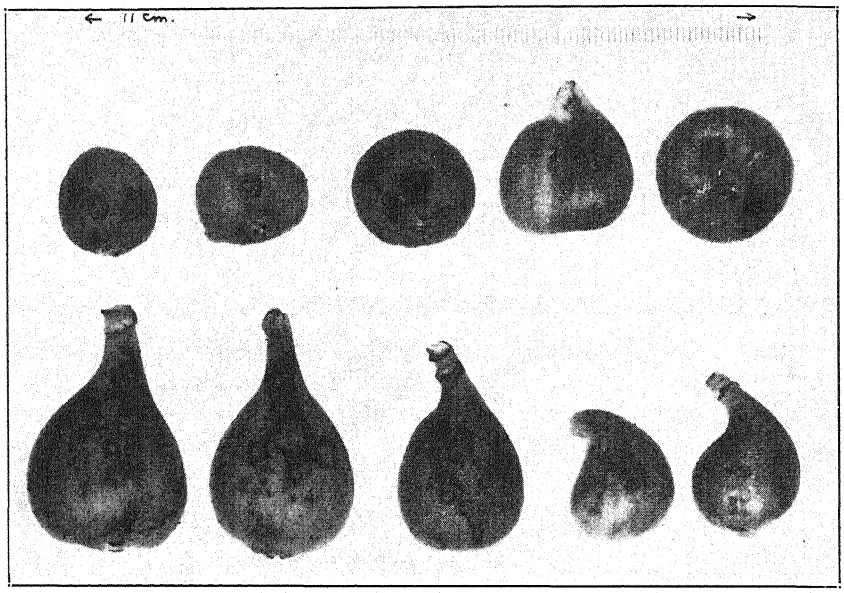


FIG. 1. Mosaic spot of the caprifig has been found only on the Samson variety and on a few seedlings; it is apparently caused by a virus.

*Caprifig Seedlings.* A few years ago, mosaic spots very much like those on the Samson were found on a seedling caprifig. Since then similar spots have been found on the profichi of 58 caprifig seedlings out of a total population of 4,034 seedlings that have been fruiting at Riverside since 1937. Most of these seedlings were the progeny of 6 common figs pollinated by Roeding No. 1 caprifig, which never shows mosaic spots. No seedling caprifigs of the cross Calimyrna × Samson showed this mosaic spotting of profichi, although it is the Samson on which the spot is so prevalent.

## The Mosaic Spot and its Effect on the Fruit

The mosaic spots on caprifigs are first manifest as watery or discolored areas on the body or neck of the fruit. These areas gradually become circular, or crescent-shape, in outline, often forming rings from 1 to 6 mm.

in diameter (Fig. 1). The most distinctive spots are circular, craterlike protrusions, depressed at the center, the whole becoming brownish in color and finally necrotic. Some spots are in the form of concentric circles, the outer circle occasionally as much as 10 mm. in diameter. Other spots are irregular in outline, brownish in color, and bordered by areas deficient in chlorophyll.

The necks of profichi appear to be very susceptible to this spotting. Frequently, the tissues on only one side of the neck are affected, and the result is a characteristically lopsided or crooknecked fruit (Fig. 1).

## Laboratory Studies

Tissues of profichi affected by spotting were prepared by the paraffin method, sectioned, and studied under the microscope.

Cells of the parenchyma are the first ones to be affected. In this tissue there appear dense aggregations of cells, often with collapsed walls, as contrasted with the spherical and normal thin-walled cells. The affected cell areas gradually enlarge until they extend from the fibrovascular bundles to the surface of the fruit, and the whole area becomes more or less necrotic.

Spotted fruits of the profichi crop of the Samson caprifig, when cultured, failed to produce any organisms that might be responsible for the spotting. No fungus hyphae were found in thin sections prepared for microscopic examination.

### MOSAIC SPOTS OF EDIBLE FIGS

No spots exactly like those found on caprifigs have been noted on edible figs, although at least 3 seedlings have shown fruit with spots somewhat comparable. These 3 seedlings are: No. 32–49, of unknown parentage; and Nos. 61–51 and 61–56, both of the progeny White Genoa × Roeding No. 1. The spots on No. 32–49 are often in concentric rings, although they appear mostly as minute pustules. As shown in figure 2, E, spots on seedling No. 61–51 are unusually prominent and appear as brown rings or as dark circular areas. The spots persist on the mature fruit and somewhat mar the normal coloration.

Mosaic spots on fruits of named varieties show a definite relation to leaf mosaic on the same tree. Thus Mission, which is often badly mosaicked in foliage, shows prominent spotting of the immature fruit (Fig. 2, A). Turkey also shows mosaicked areas, both on leaves and on fruit. On figs of these two varieties, the spots are mostly apparent as sharply defined, light-green circular areas contrasting with the normal dark-green color of the fruit. In some varieties, such as Grise St. Jean, the circular spots may become rusty or necrotic (Fig. 2, B). As previously recorded,[7] mosaic spots on fruits of Brunswick (Fig. 2, C), Celeste, and Sultane, not only cause deformation of the fruit but also bring about premature abscission of the fruit stalk.

[7] See footnote 4.

TRANSMISSION OF FIG MOSAIC

Mosaic spots on fig fruits are assumed to be caused by the same virus that causes mosaic of fig leaves. A preliminary experiment on the transmission of the disease was accordingly attempted.

Plants of tomato and of wild tobacco (*Nicotiana glauca* Graham) were inoculated with the sap from conspicuously mosaicked shoot tips and leaves of Mission fig trees. Carborundum powder was used in these inoculations, substantially as suggested by Rawlins,[8] except that the infusion was diluted

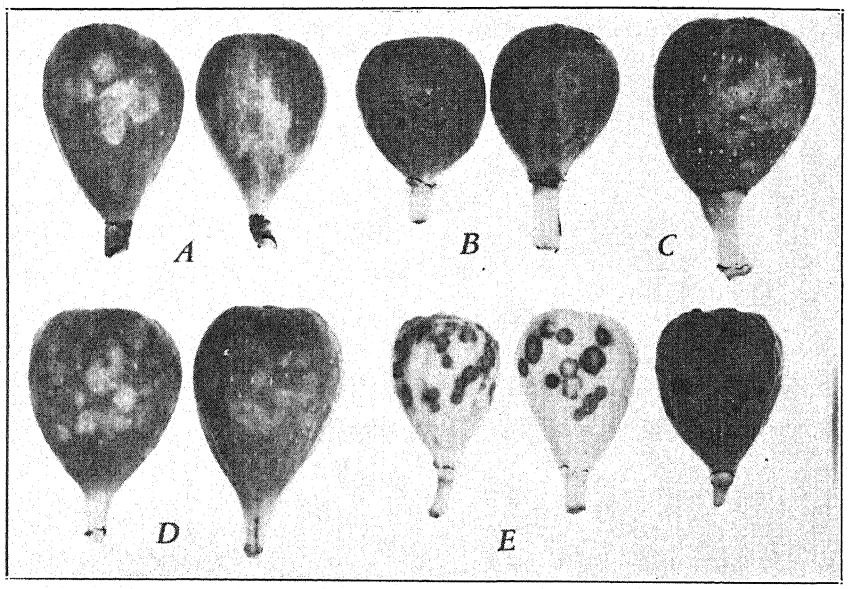



FIG. 2. Mosaic spots on edible figs. A. Mission. B. Grise St. Jean. C. Brunswick. D. Turkey. E. Seedling 61–51.

with distilled water (the amount of water being less than that of crushed tissue) and the leaves of the plants were rubbed rather vigorously with a cotton plug before inoculation. The inoculum was washed off after a few minutes by sprinkling the plants freely. Results were apparently negative. J. M. Wallace kindly assisted in examination of the treated plants.

SUMMARY AND CONCLUSIONS

Over twenty years ago Samson caprifigs of the profichi crop were found bearing blemishes or spots in the form of circular brownish protrusions. These spots were found only on the Samson and appeared on profichi of all trees of this variety examined in California. Affected figs become malformed and drop prematurely. During the past few years, similar spots have been found on 58 caprifig seedlings out of a total population of 4,034

[8] Rawlins, T. E., and C. M. Tomkins. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopath. 26: 578–587. 1936.

seedlings fruiting at Riverside. Mosaic spots of caprifigs, when cultured, failed to produce any pathogen that might be responsible for the spotting. This spotting of caprifigs is apparently the result of a mosaic disease rather narrowly restricted as to its host and somewhat peculiar in its expression.

Mosaic spots on edible figs differ somewhat from those on caprifigs. Some are in the form of concentric rings or dark circular spots, while others are apparent as sharply defined, light-green circular areas.

Attempts to transmit leaf mosaic of fig to tomato and wild tobacco were unsuccessful.

UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA.

# GROWTH SUBSTANCES AND THE RUST FUNGI[1]

DAVID GOTTLIEB AND HELEN HART

(Accepted for publication December 21, 1942)

Relationships between the fungi and growth substances have been investigated since the turn of the 20th century, but it is only as chemically pure growth substances were made available that the information has been definite and detailed. Since 1930, research on vitamin requirements of fungi has increased greatly, and a recent review of the literature by Robbins and Kavanaugh[2] lists about 350 fungus species whose vitamin relationships have been studied. The genera *Puccinia* and *Erysiphe* are not mentioned in the report of Robbins and Kavanaugh; and it is only recently that Pryor[3] reported that under certain conditions thiamin increased the development of powdery mildew on cantaloupe. Except for this work on *Erysiphe,* no information is available concerning the vitamin requirements of these obligate parasites. Naturally, one cause has been the impossibility of growing these fungi except on their natural hosts. Another may be the assumption that obligate parasites receive all the necessary vitamins or growth substances from their hosts. Not all hosts are equally suitable for growth of rusts, however. A certain rust may grow well on one host and poorly or not at all on a closely related one. For example, race 17 of *Puccinia graminis tritici* grows well on Marquis but is unable to grow to any extent on Reliance, both *vulgare* wheats. The opposite is true of race 38, which grows well on Reliance and poorly on Marquis. Although it has not been proved that wheat plants contain substances that promote growth and development of fungi, the inability of a rust race to grow within the noncongenial host may be due to absence or to insufficient amounts of proper growth substances. One variety of wheat might contain sufficient quantities of the growth substances required by many of the physiologic races of rust and would be susceptible to those races, while another variety might lack the particular growth-promoting substances needed by a certain race and as a result would be resistant to that race. It seemed possible that supplementing whatever a natural host afforded a rust parasite might enable the rust to make better progress in tissues of a host on which it grew poorly or not at all. Therefore, a number of experiments were made with 4 physiologic races of *Puccinia graminis avenae* on 3 varieties of oats and 7 races of *P. graminis tritici* on several varieties of common and durum wheat, an emmer, and einkorn.

[1] Paper No. 2054 in the Scientific Journal Series, Minn. Agr. Exp. Station.

[2] Robbins, William J., and Virgene Kavanaugh. Vitamin deficiencies of the filamentous fungi. Bot. Rev. 8: 412–471. 1942.

[3] Pryor, Dean E. The influence of vitamin $B_1$ on the development of cantaloupe powdery mildew. Phytopath. 32: 885–895. 1942.

### EFFECTS OF GROWTH SUBSTANCES ON REACTIONS OF SEEDLINGS TO RUST

Only water-soluble growth substances were used in experiments with wheat or oats seedlings and their respective stem rusts. Thiamin chloride, riboflavin, nicotinic acid, ascorbic acid, and beta-indole acetic acid were used alone, in $10^{-4}$ or $10^{-6}$ molar concentration, and a mixture of the 5 substances also was used and designated "mixture." Cereal seeds were soaked in the reagents for 24 hours preceding planting. In some experiments each 4-inch pot of seedlings received 75 cc. of the appropriate solution each day, so that the vitamins might be absorbed through the roots. It was expected that such absorbed materials would soon reach mesophyll cells of the seedling leaves and become available to the rust hyphae through their haustoria within the cells. In other experiments vitamins were supplied directly to the mycelium by filling the interstices of the leaf tissue with the solution. A hypodermic syringe fitted with a short, thick-walled rubber tube in place of a needle, as Newton, Lehman, and Clark[4] suggested, was placed against the leaf surface and the vitamin solution was gently forced through the stomata until the interstices were filled and about 2 inches of the tissue on both sides of the rubber tube appeared water-soaked. With slow and gentle pressure, no injury resulted. The water-soaked appearance lasted only a short time, usually less than an hour; but during that time the solution and rust mycelium were in direct contact. In still other experiments rusting seedling leaves were supplied with vitamin solutions by a method used by Gassner and Hassebrauk.[5] Pots of rusting seedlings were inverted over solutions of growth substances, and the leaves remained submerged for several hours at a time to permit a certain amount of absorption through the leaf. Table 1 summarizes the different seedling experiments. Throughout the series there were no consistent changes in development of rust or in seedling host reaction to rust that could be attributed to the treatments with growth substances. There were minor differences in the number of hours required for flecking, in the degree of chlorosis of infected tissues, and even in the abundance of sporulation. The reactions of Khapli emmer to race 15 of *Puccinia graminis tritici* ranged from flecks to type 1 and those of Marquis to its virulent races ranged from type 3 to type 4. Nevertheless, the variations were not consistent in duplicate pots of seedlings nor in repeated experiments. Most of the variations from the checks were within the range of variability normally attributed to slight differences in environment. No host that was normally resistant to the rust race involved became susceptible to that race after vitamin treatment; and there was no definite suppression of rust in hosts that were normally susceptible to the rust race used. The

[4] Newton, R., J. V. Lehman, and A. E. Clark. Studies on the nature of rust resistance in wheat. Can. Jour. Res. (C) 1: 5–35. 1929.

[5] Gassner, G., and K. Hassebrauk. Über die Beeinflüssung der Rostanfälligkeit durch Eintauchen geimpftes Blätter in Lösungen von Mineralsalzen und anderen Stoffen. Phytopath. Zeitschr. 5: 323–342. 1933.

water-soluble growth substances apparently exerted no influence on stem rust in wheat and oats seedlings.

*Detrimental effects of growth substances.* Generally there was very little effect on host or on parasite when the growth substances were supplied to cereal seedlings in different ways. In certain cases, however, nicotinic acid injured wheat seedlings. When a $10^{-4}$ molar solution was used to fill the intercellular spaces by means of the rubber-fitted syringe, tip burning was

TABLE 1.—*Varieties of wheat and oats inoculated as seedlings with different physiologic races of stem rust and supplied in various ways with growth substance*

| Physiologic race of rust | | Host variety | Means of supplying growth substances | Reaction[a] to rust |
|---|---|---|---|---|
| *Puccinia graminis avenae* | 5 | Oats<br>Bond | Solution forced into leaf interstices | S |
| | 5 | Minrus | do | R |
| | 8 | Bond | Solution absorbed through roots | S |
| | 8 | Seven-o-three | do | S |
| | 10 | Bond | do | S |
| | 10 | Seven-o-three | do | R |
| | 11 | Bond | do | S |
| | 11 | Seven-o-three | do | R |
| *P. gr. tritici* | 15 | Khapli emmer | Solution forced into leaf interstices | R |
| | | Wheats | | |
| *P. gr. tritici* | 15 | Marquis | Solution forced into leaf interstices | S |
| | 17 | do | Solution absorbed through roots | S |
| | 17 | Reliance | do | R |
| | 38 | Marquis | do | R |
| | 38 | Reliance | do | S |
| | 56 | Arnautka | Solution forced into interstices | R |
| | 56 | Mindum | Leaves immersed in solution | R |
| | 56 | Kubanka | do | R |
| | 56 | Einkorn | Leaves immersed in solution | R |

[a] Reaction to rust in all these experiments was the same as the reaction expected with each host-parasite combination under normal conditions. S denotes susceptibility; R denotes resistance.

severe on both Arnautka and Marquis wheats. Slight burning occurred when the same concentration of nicotinic acid was furnished for root absorption by wheat seedlings of several varieties. Injury to the host was never noted with any of the other growth substances.

## RUST DEVELOPMENT ON OLDER PLANTS

Forcing a urediospore suspension into unexpanded portions of a cereal culm provides an ideal incubator for spore germination and infection of young and succulent host tissues. The severity of infection resulting is high, except when the leaf sheaths are too tightly rolled to permit introduction and distribution of the spore suspension. Urediospores of certain rust races were suspended in vitamin "mixture" solution and then introduced by hypodermic syringe and needle into various parts of culms of field-grown wheat varieties (Table 2). Similar inoculations with urediospores suspended in distilled water served as checks. By providing growth substances

for absorption by the fungus germ tubes, it might be possible to augment the initial vigor of the infecting rust, so that the rust could more easily and quickly parasitize the noncongenial host. Actually, however, no differences in rust development or host reaction were due to the suspension medium. Older parts (leaf sheaths, peduncles, glumes, and awns) of wheat varieties that were susceptible as seedlings to the races of rust with which they were inoculated remained susceptible. Conversely, those that were resistant or immune as seedlings remained resistant or immune under the two types of

TABLE 2.—*Field-grown varieties of wheat inoculated in boot or in a preboot stage with urediospores of different physiologic races of stem rust suspended in solutions of growth substances*

| Physiologic race of rust | Host variety | Stage of host inoculated | Reaction[a] to rust |
|---|---|---|---|
| *Puccinia graminis tritici* 15 | Marquis | Preboot | V.S. |
| | | Boot | S. |
| 15 | Thatcher | Preboot | M.S. |
| | | Boot | M.S. |
| 15 | Hope × Thatcher hybrid | Preboot | V.S. |
| | | Boot | M.S. |
| 17 | Ceres | Preboot | M.S. |
| | | Boot | S. |
| 17 | Thatcher | Preboot | I. |
| | | Boot | I. |
| 19 | Marquis | Preboot | M.S. |
| | | Boot | M.S. |
| 19 | Coronation | Preboot | M.S. |
| | | Boot | M.S. |
| 34 | Hard Federation | Preboot | V.S. |
| | | Boot | V.S. |
| 34 | Hope | Preboot | V.R. to S. |
| 38 | Marquis | Preboot | M.R. |
| | | Boot | M.S. |
| 36 | Ceres | Preboot | V.S. |
| | | Boot | V.S. |
| 38 | Coronation | Preboot | M.S. |
| | | Boot | M.S. |

[a] V.S. = very susceptible.
S. = susceptible.
M.S. = moderately susceptible.
M.R. = moderately resistant.
V.R. = very resistant.
I. = immune.

inoculation. Thus, in the field and on older plants, as well as on seedlings, the growth substances used had no effect on stem rust development.

### GERMINATION OF SPORES IN GROWTH SUBSTANCE SOLUTION

To determine the effect of growth substances on the germination of rust spores, agar blocks were impregnated with the vitamin solution. Loegering[6] first used relatively large surfaces of 2 per cent water agar to study germinating rust spores, and Mitchell[7] cut blocks of agar and allowed different chemical reagents to diffuse through them so that he could study the effects on germination of rust spores. From hardened 2 per cent agar, blocks $\frac{3}{4}$

[6] Loegering, William Q. A satisfactory medium for germination of urediospores of *Puccinia graminis tritici*. Phytopath. 31: 952–953. 1941.

[7] Unpublished results of experiments of J. E. Mitchell, Minnesota Agricultural Experiment Station.

inch square and $\frac{1}{8}$ inch thick were cut, then placed in vitamin solution for 24 hours so that the reagents might diffuse through the agar. Impregnated blocks, with excess solution removed by filter paper, were put on glass slides, and urediospores were spread over them before they were placed in a moist chamber. Freshly collected viable stem rust urediospores and also urediospores stored at low temperatures for several weeks or months were used, and spore germination was observed at regular intervals. Within spore lots there were no significant differences in the germination of spores spread over the surface of a plain agar block or spread over the surface of an agar block through which the vitamin "mixture" had diffused. Percentages of spores germinating, time required for germination, and length and vigor of germ tubes were approximately the same in all replicates of a single spore lot.

## CONCLUSIONS

From the general negative results of the foregoing experiments it is concluded that the water-soluble growth substances, thiamin chloride, riboflavin, nicotinic acid, ascorbic acid, and beta-indole acetic acid, do not play an important role in the problem of resistance in cereal plants to the obligately parasitic stem rust; and that pathogenic differences in physiologic strains within a rust species may not be attributed to different growth substance requirements.

UNIVERSITY FARM,
ST. PAUL, MINNESOTA.

# A NONCHROMOGENIC SPORULATING VARIANT OF ALTERNARIA SOLANI[1]

H. Rex Thomas

(Accepted for publication January 28, 1943)

*Alternaria solani* (E. and M.) J. and G., as most frequently isolated in Indiana from tomatoes afflicted with early blight, produces a yellowish to deep-red pigmentation in potato-dextrose agar and sporulates sparingly. A culture, designated S–16, which does not color the agar and produces conidia profusely, arose as a variant from a culture obtained from a single-spore culture obtained from an early-blight lesion on an infected tomato leaf collected at Santa Maria, California, by M. W. Gardner in 1937.

The culture has been very useful in laboratory, greenhouse, and field studies of early blight wherein the use of large numbers of conidia was necessary. It has been found difficult to produce conveniently an adequate number of conidia on the typical *Alternaria solani* cultures by application of methods[2,3,4,5] reported to stimulate sporulation. The S–16 culture also has been useful as a marker in studies designed to determine the role of inoculum introduced on southern-grown tomato seedlings in initiating or increasing the severity of early-blight infection in Indiana tomato fields as distinguished from that inoculum native in such fields.

Such studies were made by atomizing locally grown tomato seedlings, at time of pulling, with a dilute suspension of conidia from the S–16 culture, packing the plants as is done commercially, and storing them for 48 hours before setting. The seedlings became infected in a manner similar to southern-grown seedlings shipped north after becoming contaminated with early-blight conidia at the time of pulling and packing. Near the close of the tomato season, isolations were made from early-blight leaf spots, stem cankers, and infected fruits to determine what proportions were traceable to the S–16 and to native field inocula, respectively.

Bonde[6] has shown that strains of *Alternaria solani* exist on the potato, and that many strains may develop through saltation in culture. During the winter of 1937–1938 several hundred Alternaria conidia were collected on tomato débris in southern, central, and northern Indiana and from aerial spore traps located at Vincennes and at Lafayette, Indiana. Representative

[1] Cooperative investigation of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, United States Department of Agriculture and the Department of Botany, Purdue Agricultural Experiment Station. Journal Paper No. 75, Purdue University Agricultural Experiment Station.

[2] Dillon Weston, W. A. R. The sporulation of *Helminthosporium avenae* and *Alternaria solani* in artificial culture. Trans. Brit. Mycol. Soc. **20**: 112–115. 1936.

[3] Kunkel, L. O. A method of obtaining abundant sporulation in cultures of *Macrosporium solani* E. and M. Mem. Brooklyn Bot. Gard. **1**: 306–312. 1918.

[4] Ramsey, G. B., and Alice A. Bailey. Effect of ultra-violet radiation upon sporulation in Macrosporium and Fusarium. Phytopath. (Abstract) **20**: 141. 1930.

[5] Rands, R D. The production of spores by *Alternaria solani* in pure culture. Phytopath. **7**: 316–317. 1917.

[6] Bonde, Reiner. Physiological strains of *Alternaria solani*. Phytopath. **19**: 533–548. 1929.

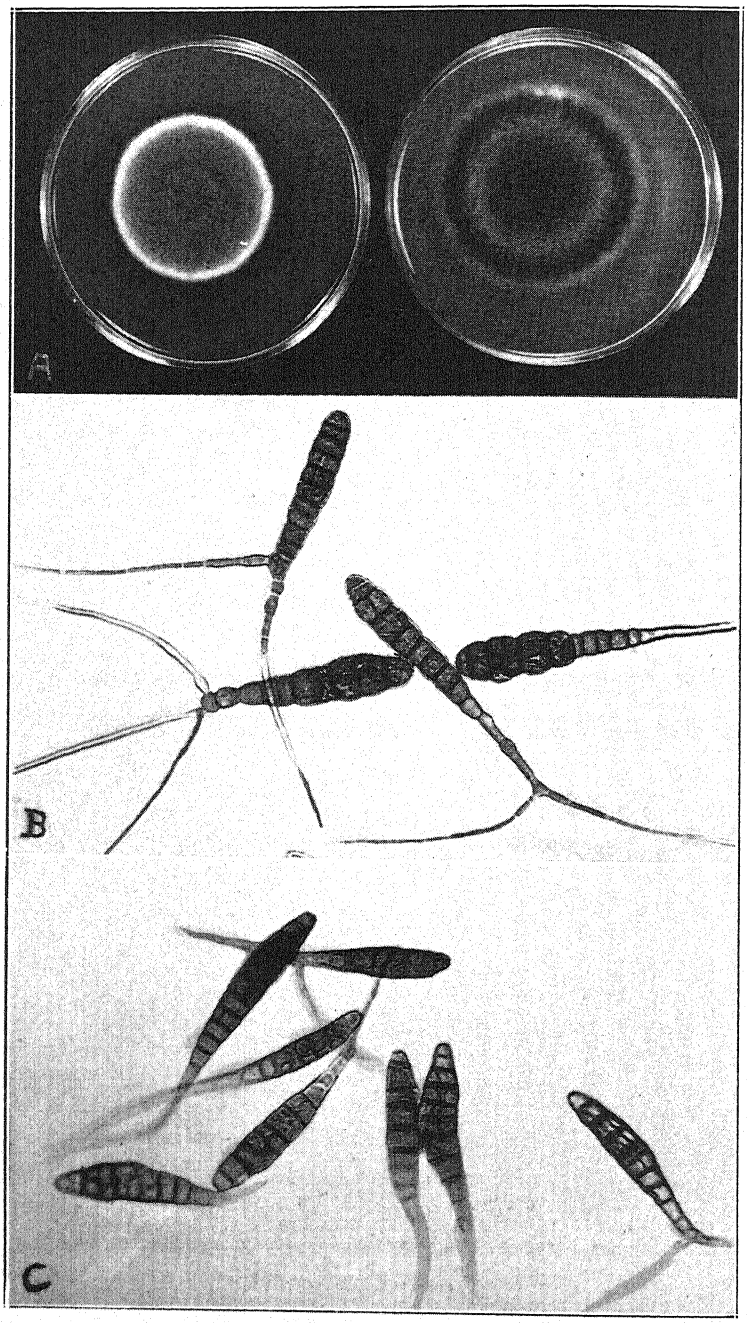



FIG. 1. A. Five-day-old culture on potato-dextrose agar of variant S–16 at left, and of typical chromogenic, sparsely sporulating *Alternaria solani* isolate at right. B. Conidia from S–16 culture. C. Conidia from sparsely sporulating, typical *A. solani* culture.

cultures obtained from these conidia were tested for pathogenicity by determining if they produced lesions at the base of the stems of young tomato seedlings. Only those cultures that were chromogenic were found pathogenic. No culture approaching S–16 in the production of conidia was isolated from the field. Variants have arisen frequently in *A. solani* cultures but no attempt was made in this work to segregate and study them. To date it has been possible to maintain S–16 as a nochromogenic, sporulating culture by mass transfer.

On potato-dextrose agar, S–16 grows more rapidly, has a darker surface color, and has a more diffuse type of growth than the usual isolate. Conidia may develop on 3-day-old cultures and on mycelium that is only 24 hours old. Plate cultures 8 to 10 days old have yielded the maximum number of spores.

Adequate comparison of the conidia from S–16 and with those from typical cultures have not been made because of insufficient numbers of conidia from the chromogenic cultures. In limited observations conidia from the S–16 culture have appeared somewhat the larger in culture. The following measurements of conidium length do not include the beak, as the length of the beak differs greatly in conidia that are of approximately the same size otherwise. Of 25 conidia of S–16 obtained on artificially inoculated tomatoes in the field, the mean length was $72.2 \pm 2.0$ μ and the mean width was $17.7 \pm 1.9$ μ. The mean lengths of 3 samples of 100 *Alternaria solani* conidia, collected on naturally infected tomatoes in the field, were $83.3 \pm 1.0$ μ, $80.5 \pm 1.8$ μ, and $94.4 \pm 1.4$ μ. The mean widths varied from $17.3 \pm 0.3$ μ, $16.8 \pm 0.3$ μ, and $17.0 \pm 0.3$ μ. Since the different collections of *A. solani* conidia may vary considerably in size, it would seem that the S–16 culture probably is not significantly differentiated in size from the general population.

Collections of *Alternaria solani* conidia obtained in nature vary greatly in the proportionate occurrence of those with forked beaks. In 3 collections of conidia of *A. solani* studied, 2 were found to contain no fork-beaked conidia in 100 observed, while in the third collection 62 per cent were fork-beaked. In 100 conidia of variant S–16 from culture, 61 per cent were fork-beaked, while none of 25 conidia from the field were fork-beaked.

The pathogenicity of a conidial suspension of variant S–16 from culture was compared with that of a suspension of conidia of *Alternaria solani* collected in the field. A dilute suspension of conidia was placed in drops on tomato foliage after which the plants were kept moist for 72 hours. Infection occurred at all points of inoculation with each conidial suspension. No difference in the size of the leaf spots could be observed. The S–16 culture has produced stem lesions, leaf spots, stem-end rot of the fruit, and spotting of the green fruit in the field, similar to that produced by typical *A. solani*. In the field the S–16 variant has not been observed to sporulate more abundantly than other isolates of *A. solani*.

PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION,
LAFAYETTE, IND.

# CHLAMYDOSPORE GERMINATION IN THE FUNGUS CAUSING DWARF BUNT OF WHEAT[1]

C. S. HOLTON[2]

(Accepted for publication January 12, 1943)

## INTRODUCTION

Dwarf bunt of wheat, caused by a peculiar race of *Tilletia tritici* (Bjerk.) Wint., is distinguished from the more common races of *Tilletia* spp. on wheat by certain striking symptoms and characteristics.[3] One of these is the apparent nonviability of the chlamydospores of the fungus under laboratory conditions, which also is reflected in the failure to obtain infection by seed inoculation. Recently it was reported[3] that certain hyaline, haploid cells associated with the chlamydospores were capable of germination, but it was pointed out that these viable cells were not present in sufficient numbers to perpetuate the disease in destructive proportions, unless the mycelium produced by them had unusual capacity for vegetative propagation in the soil. However, this possibility has been eliminated by the results of experiments (not yet reported) proving that the mycelium of the dwarf bunt fungus develops perhaps even less extensively in soil than does that of the ordinary bunt fungi. Therefore, further attempts to germinate the chlamydospores have been made, and the results are reported in this paper.

## MATERIAL AND METHODS

The chlamydospores of the dwarf-bunt fungus used in these tests were collected from time to time in commercial wheat fields in 13 areas of infestation in the western wheat region. The spores of the various collections were stored at room temperature from 1 to 6 years, depending upon when the collections were made. All germination tests reported were made in 1942.

As already reported,[3] the various treatments used in earlier experiments on the dwarf-bunt chlamydospores failed to induce germination. In more recent experiments, however, the spores were soaked in tap water[4] for 1 to 7 months while stored in an electric refrigerator kept at about 4° C. The water was changed about once a month to reduce contamination.

Germination tests were made on 2 per cent water agar in Petri dishes. The period of incubation extended to 2 months at 3 temperatures approximating 5, 10, and 20° C. Observations to determine whether there was spore germination were made weekly, beginning with the first week of incubation.

[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, United States Department of Agriculture, and the Washington Agricultural Experiment Station.

[2] Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry.

[3] Holton, C. S. Preliminary investigations on dwarf bunt of wheat. Phytopath. 31: 74–82. 1941.

[4] Suggested by the fact that G. W. Fischer observed germination of apparently nonviable spores of certain species of *Tilletia* from grasses after the spores had been soaked in water one month.

RESULTS

The chlamydospores of all the collections tested germinated in percentages ranging from a trace to as high as 30 per cent, the highest percentages occurring at 10° C.

Perhaps more notable than the percentage of germination was the characteristically branched type of promycelium that was produced by the germinating spores (Fig. 1). The number of branches varied from 2 to 4 and the branching occurred at various points on the promycelium from the base (Fig. 1, J) to the tip (Fig. 1, E and F). Branches may arise laterally on the main promycelium (Fig. 1, C, I, and H) or else the main promycelium may divide at the tip and produce branches of similar dimensions (Fig. 1,

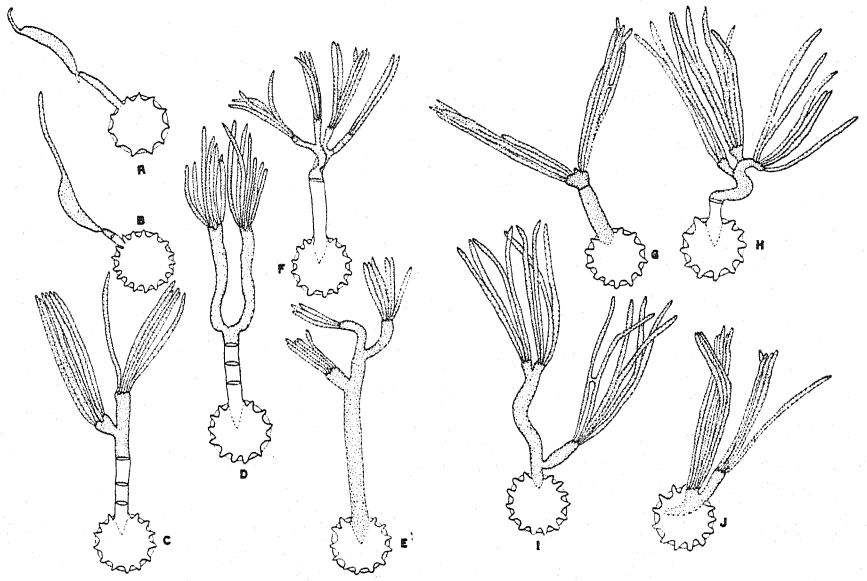



FIG. 1. Freehand drawings of germinating chlamydospores of the dwarf-bunt fungus. A and B. Haploid type of germination. C, H, and I. Lateral branches produced from the main promycelium. D. The main promycelium divided at the tip to produce two branches of similar dimensions. E. The main promycelium produced a lateral branch and then divided at the tip to produce two more branches. F. The promycelium divided at the tip resulting in two branches, each of which also divided at the tip, thus forming four branches. G. Two distinct groups of sporidia produced on the enlarged end of the promycelium. J. The main promycelium produced a lateral branch from its base. (Note the slight degree of bifurcation at the tips of branches in C, D, and I.)

D). Some of the branches appear to be septate (Fig. 1, F). Less frequently observed is the type shown in figure 1, G, in which two distinct groups of primary sporidia are borne on the enlarged end of the promycelium. Although not illustrated, unbranched promycelia were produced by many of the spores.

The germination process illustrated in figure 1, A and B, is similar to that already described for the hyaline cells.[5] Instead of a promycelium a

[5] See footnote 3.

sterigma is produced bearing a so-called secondary or sickle-shape sporidium, thus indicating that these chlamydospores are haploid. While this type of germination does not occur commonly, it has been observed on numerous occasions, suggesting that the presence of a limited number of these spores in dwarf-bunt collections may be regarded as typical.

The number of primary sporidia produced on each promycelial branch ranged from 2 to 8 (Fig. 1, F and J), and the total on any one promycelium ranged from 9 to 16 (Fig. 1, G and F). The length of the sporidia on certain promycelia varied considerably (Fig. 1, D and G), but whether this was due to different stages of maturity or to some other factor could not be determined. The primary sporidia fused in pairs (Fig. 1, G and I) in the same manner as is characteristic of the species. Apparently, most of the sporidia illustrated in figure 1 had not yet developed to the fusion stage. Unlike those of other races of the bunt fungi, the fused sporidia (H-shape structures) of the dwarf-bunt fungus rarely produced secondary sporidia. Instead, a mycelium was produced directly from the fusion. It is assumed that this mycelium was the infection hypha. On the other hand, secondary sporidia were produced in abundance in culture by certain of the monosporidial lines derived from the germinating hyaline spores,[6] thus indicating genetic differences for this behavior. The type of structures produced by the germinating spores may have some bearing on the classification of the dwarf-bunt fungus.

Whether the differences in viability of spores of different collections were due to inherent factors or to environmental influence has not yet been determined. However, in certain collections the percentage germination increased with prolonged soaking. For example, spores of one collection that had soaked 11 weeks germinated less than 5 per cent after 18 days' incubation at 10° C. After soaking for 4 months, these spores germinated 15 per cent in 8 days and after soaking 5 months they germinated 25 per cent in 4 days. Similar results were obtained with the spores of several other collections, whereas still other collections failed to respond to increased periods of soaking. However, the amount and degree of branching of the promycelium appeared to be reduced in spores that had soaked 5 months or more. Thus, while there were definite indications that increased periods of soaking increased the percentage germination, such increased soaking seemed to decrease the amount and degree of branching.

Whether the facts revealed by these observations on germination of dwarf-bunt chlamydospores are significant in relation to the natural occurrence of this disease would seem to be questionable, since, under field conditions, the spores are not subjected to a prolonged period of soaking in water immediately prior to the time when infection occurs. Under field conditions the spores, as well as the soil, are subjected to alternate wetting and drying and freezing and thawing, but these conditions in the laboratory failed to

[6] See footnote 3.

induce germination.[7] Nevertheless, the demonstrated fact that dwarf-bunt chlamydospores are viable and can be induced to germinate by a relatively simple method undoubtedly will lead to more effective investigation of this problem.

SUMMARY

Chlamydospores of the dwarf-bunt fungus were induced to germinate by prolonged soaking (3 months or more) in tap water kept at about 4° C. Germination was relatively low, ranging from a trace to about 30 per cent in different collections.

Most of the germinating spores produced a branched promycelium, the number of branches ranging from 2 to 4. Two to 8 primary sporidia were borne on each branch and some of these sporidia fused in pairs. A fused pair of sporidia usually produced a mycelium, which presumably was the infection hypha. Relatively few sickle-shape secondary sporidia were developed.

The type of structures produced by the germinating spores may have some bearing on the classification of the dwarf-bunt fungus.

DEPARTMENT OF PLANT PATHOLOGY,
WASHINGTON STATE COLLEGE,
PULLMAN, WASH.

[7] See footnote 3.

# CONTROL OF PITHIUM ROOT ROT OF ALOE VARIEGATA BY HOT-WATER TREATMENT

KENNETH F. BAKER AND KATHARINE CUMMINGS

(Accepted for publication November 20, 1942)

## INTRODUCTION

In a local nursery several thousand seedling plants of *Aloe variegata* L. were discarded because of a root rot induced by *Pythium ultimum* Trow.[1] and possibly other Pythiaceae. The decay usually began at the root tip, and in a short time progressed back to the stem. Under commercial conditions of growth the final result was a stunted top with pallid, hard leaves and no roots (Fig. 1, A, B). However, if plants were kept quite moist the fungus advanced into the stem and leaves, reducing them to a soft, watery

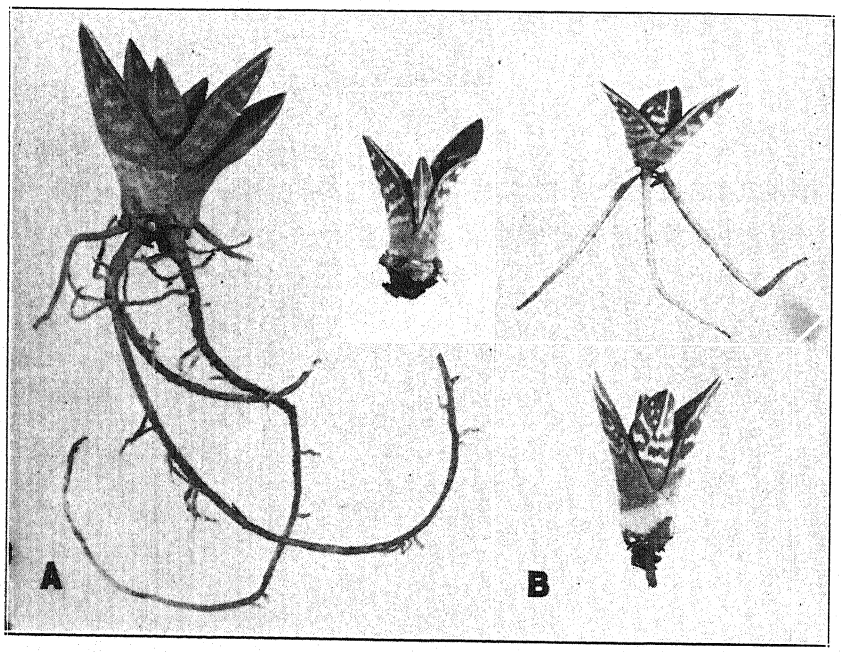



FIG. 1. Control of Pythium root rot of *Aloe variegata* by hot-water treatment at 46° C. for 20 minutes. A. Treated plant at left and check at right, 100 days after planting. B. Treated plant above, and check below, 20 days after planting; the former showing rapid recovery.

mass inside of the heavy epidermis, and soon decayed the whole plant. Since 2 to 3 years are required to produce plants for the market, the loss is considerable under the prevalent practice of discarding diseased seedlings.

The plant withstands relatively high temperatures in its native habitat in Karoo and Namaqualand, South Africa. This fact together with the

[1] Identified by John T. Middleton.

relatively low thermal tolerance of most Pythiaceae, suggested a method of heat treatment to salvage plants with decayed root systems.

The use of heat to destroy pathogens in living plant tissue was introduced 61 years ago by Jensen (4) when he treated seed-potato tubers in a water-jacketed chamber for 4 hours at 40° C. to destroy the internal mycelium of *Phytophthora infestans* (Mont.) de Bary. This apparently was not practiced on a field scale, and Jones, *et al.* (5) later found no practical field benefits from its use in Vermont. Several subsequent investigators have used similar treatments against other Pythiaceae in living vegetative structures. Rosenbaum (9) found that immersion of green tomato fruits in water at 60° C. for 1½ minutes killed the mycelium of *Phytophthora parasitica* Dast. (*P. terrestris* Sherb.) in non-visible surface lesions. Fawcett (2) was able to destroy the mycelium of *P. citrophthora* (Sm. and Sm.) Leon. in lemon fruits by holding them in hot water for 2 minutes at 48.9° C. within 30 hours after infection, or at 46.1° C. within 8 hours after infection. In 1926 Murphy and McKay (6) found that dry air at 40° C. for 8, 16, or 24 hours, or at 38° C. for 16 hours killed the mycelium of *Peronospora schleideni* Unger without injury to onion bulbs. Brown (3) reported good control of crown and root rot of peony after removal of diseased areas and treatment in water at 48.9° C. for 30 minutes; *Phytophthora cactorum* (L. and C.) Schroet. (*P. paeoniae* C. and P.) was among the organisms involved. Ark and Barrett (1) treated asparagus spears infected with *Phytophthora* sp., killing the organism by immersion in hot water at 46° C. for 1 minute.

## METHODS AND RESULTS

In this study a total of 505 plants of *Aloe variegata,* 1, 2, and 3 years old, were dried for several days in the laboratory and then immersed in water of varying temperatures to determine the heat sensitivity of the host and parasite. The plants were those actually discarded because of root rot by the nurseryman and were selected only to the extent of eliminating a few with rotted tops. Wire test-tube baskets with cheesecloth tops were used to hold the seedlings during immersion. After treatment the plants were spread out to cool and dry before being planted in pasteurized light sandy loam; the checks were planted in other containers of the same soil. Some were planted singly in 3-inch pots and some spaced about 1½ inches apart in flats. The soil was kept very wet and the plants held in a cool, humid greenhouse, conditions most favorable for the development of the disease and apparently so recognized by growers (7, 8).

Three tests were made at different times using, variously, water at 40.5, 43, 46, 49, 51.5, 54.5 and 57.5 degrees C. for 20 minutes, 46° C. for 30 minutes, and 46° and 49° C. for 40 minutes. Temperatures lower than 46° did not consistently kill the pathogen, and those of 49° C. and above frequently were injurious to the Aloes. The treatment at 46° for 20 or 30 minutes eradicated the fungus without injury to the host, but the 40 minute period produced some subsequent drying of the outer leaves and might be

regarded as excessively damaging. The treated plants very quickly formed new roots which in 20 days were in some cases 2 inches long, whereas the checks had been unable to produce new roots (Fig. 1, B). The difference was even more pronounced after 100 days (Fig. 1, A).

Under commercial conditions, the use of a water treatment at 46° C. for 30 minutes would be advisable, but this may be reduced to 20 minutes for very small plants. If large specimens, such as seed-bearing plants, are to be treated the time might be extended safely to 40 minutes. The precaution of replanting the seedlings in soil freed of the pathogen by pasteurization or other means should be observed.

Limited tests with *Haworthia attenuata* Haw. gave results comparable to the above. Indeed, it seems probable that many of the cacti and succulent plants would tolerate the treatment and that salvage of plants now discarded might be economically effected.

## SUMMARY

*Pythium ultimum* causes an important root rot of young nursery plants of *Aloe variegata* in California. Because heat treatment of the plants will free them of the pathogen, the prevalent costly practice of discarding infected plants is unnecessary. A hot-water treatment of infected plants at 46° C. for 20 to 40 minutes, depending on the size of the plants, kills the parasite without injury to the host. It is essential that treated material be replanted in soil free of the pathogen.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
LOS ANGELES, CALIFORNIA.

## LITERATURE CITED

1. ARK, P. A., and J. T. BARRETT. Phytophthora rot of asparagus in California. Phytopath. **28**: 754–756. 1938.
2. BARRETT, J. T., and H. S. FAWCETT. Withertip, tear-stain and control of brown rot. Calif. Citrograph **7**: 232–233, 254. 1922.
3. BROWN, N. A. Method of control for crown and root rot of peonies. Amer. Peony Soc. Bull. **55**: 3–6. 1933.
4. JENSEN, J. L. Moyens de combattre et de détruire le Peronospora de la pomme de terre. Mém. Soc. Nat. d'Agr. de France **131**: 31–156. 1887.
5. JONES, L. R., N. J. GIDDINGS, and B. F. LUTMAN. Investigations of the potato fungus *Phytophthora infestans*. Vermont Agr. Exp. Stat. Bull. 168. 100 pp. 1912.
6. MURPHY, P. A., and R. MCKAY. The downy mildew of onions (*Peronospora schleideni*), with particular reference to the hibernation of the parasite. Sci. Proc. Royal Dublin Soc. (n.s.) **18**: 237–262. 1926.
7. OLIVER, G. W. Aloe. *In* L. H. Bailey's *Standard Cyclopedia of Horticulture* **1**: 255–256. Macmillan Co. (New York). 1937.
8. Reynolds, G. W. Liliaceae. *In* S. E. Haselton's *Succulents for the Amateur*. Abbey Garden Press (Pasadena). Pp. 149, 151. 1939.
9. ROSENBAUM, J. Infection experiments on tomatoes with *Phytophthora terrestria* Sherb. and a hot water treatment of the fruit. Phytopath. **10**: 101–105. 1920.

## PHYTOPATHOLOGICAL NOTES

*The Production of Coacervates.*—Coacervates of organic compounds often characterize pathological conditions in plant cells. The coacervates generally consist of refringent, spherical masses of phenolic material, each with a lipoidic envelope. It seems probable that these bodies are formed as a result of the disturbance of hydrogen bonds in the catechol-water system by the activity of a catechol oxidase. Dufrenoy and Reed[1] showed that the main factor in the production of the auto-complex coacervation of a phosphatid is a certain stage of desolvation. The presence of catechol or other polyphenol dispersed in the cell vacuole may act as a desolvant agent.

This note will discuss the results of experiments *in vitro* on coacervate production with chemicals. Lecithin was stained in a solution of Sudan III in methylal to make it identifiable under the microscope.

The methylal solution of Sudan III has proved to be an exceedingly useful reagent for staining the above mentioned spherical inclusions in the cell vacuole, following the technique earlier given by Dufrenoy and Reed.[2] A few cc. of this lecithin-Sudan III solution were added to the surface of a solution of catechol in a buffer solution having a reaction of pH 5.35. At the end of three hours, it was evident that the lecithin had assumed a finely divided condition. Under the microscope, one could see many globular masses, resembling coacervates (Fig. 1), each enveloped in a vesiculated

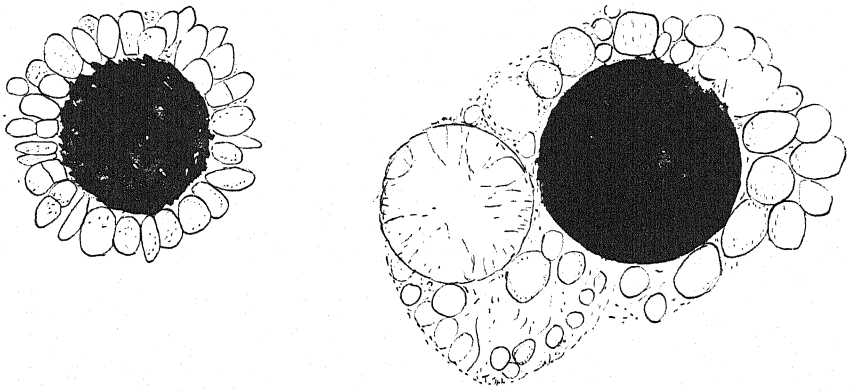


FIG. 1. Coacervates of catechol, each surrounded by a vesiculate envelope of lecithin.

layer of lecithin (identified by its orange-red color). De Jong and Hartcamp[3] observed similar hyaline vesicles on Paramecia, which had been exposed to certain chemicals that affected the solvation of the cell constituents.

The vacuolate nature of the lipoid envelope, which contains water as well as lecithin, was demonstrated in hypoplastic plant cells of orange roots that had grown in a solution lacking micro-elements.[4] Similar coacervates were

[1] Dufrenoy, J., and H. S. Reed. Coacervates in physical and biological systems. Phytopath. 32: 568–579. 1942.

[2] ———, and ———. A technic for staining cells with Sudan III in a water phase. Stain Tech. 12: 71–72. 1937.

formed in solutions of catechol buffered at pH 6.35 and pH 7.35, though smaller, and often with radiating strands of lecithin. The recent discovery that plants suffering a deficiency of micro-elements may contain coacervates of pyridoxin[5] gives point to the investigation of pyridoxin-lecithin complexes *in vitro*. The photomicrographs (Fig. 2) show coacervates produced in

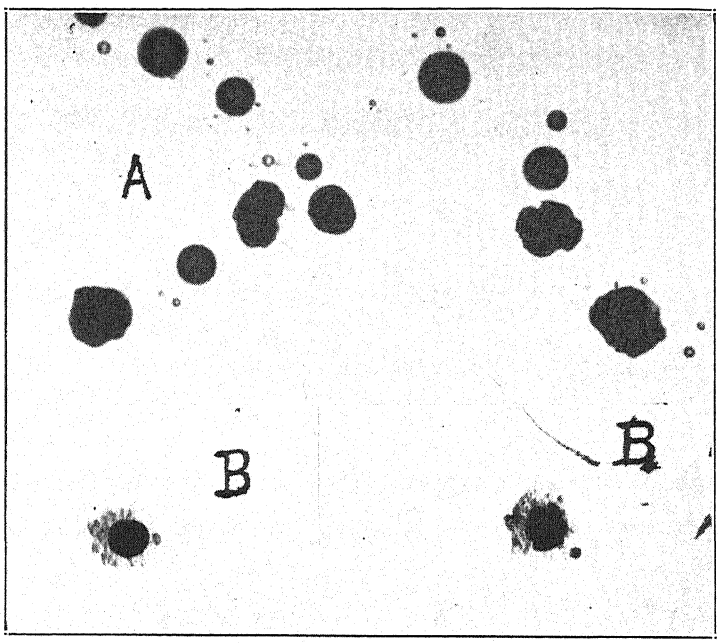



FIG. 2. Photomicrographs of coacervates of pyridoxin with lecithin, ×1290. A. Coalescence into auto-complex coacervates was evident. B. Vesiculate envelopes of lecithin surrounded the larger masses. Photographs by Dr. R. M. Reeve.

buffer solutions at pH 5.35 by the technique above described. The tendency for small coacervates to coalesce and to form larger ones, observable *in vivo*, is seen in the illustration.

I experimented also with resorcinol and hydroquinone. Coacervates were obtained with resorcinol dissolved in water, but when dissolved in a phosphate buffer solution, it did not form coacervates with lecithin. Solutions having a pH of 5.35 and 7.35 were tried, but yielded only droplets of red-stained oil, surrounded with halos of vacuolated, hydrated lecithin. In this case the resorcinol was probably not sufficiently oxidized to change the electric charge on the micelles of lecithin. Hydroquinone did not produce real auto-complex coacervates when 2 per cent solutions were placed in contact with methylal containing lecithin. In a phosphate buffer of pH 7.35 the solution turned brown after 2 days, but yielded no agglomerations resembling coacervates.

I repeated the experiments, using different solvents for lecithin, namely, chloroform and toluol, without obtaining coacervates. Good results were

obtained, however, by using alcohol as a lecithin solvent and gently placing a layer of it on top of a solution of catechol + neutral red in a phosphate buffer at pH 5.35. The alcohol solution mixed slowly with the aqueous system and allowed time for desolvation and coacervation.

The relation of coacervates to health and disease has been discussed in the works here cited. This note describes aggregates that were produced with chemicals in a simple system *in vitro,* thereby contributing information on the formation of coacervates and certain intra-cellular activities.—HOWARD S. REED, University of California, Berkeley, California.

*Breaking in Color of Flowers of Annual Phlox Caused by the Aster-yellows Virus.*—In 1938 one annual phlox plant (*Phlox drummondii*) showing breaking in color of the flowers was found on the campus of the University of California at Berkeley. All attempts to transmit the virus from diseased to healthy annual phlox grown from seeds by juice inoculations using the carborundum method were failures.

It was suspected that the virus causing the breaking in phlox was celery calico, which commonly induces breaking in pansies and violas in California. The virus extract from a number of host plants of celery calico inoculated in phlox failed to induce breaking. This virus is transmitted by 10 species of aphids, but all aphid inoculations to healthy phlox were negative.

Another virus that causes breaking in the color of pansies, violas, petunia, and ranunculus is western cucumber mosaic, which occurs in the interior regions of California. Juice and aphid inoculations failed to induce breaking in the color of annual phlox, even though it was infected with this virus. The flowers were dwarfed with rolled petals, and sometimes the corollas were dead.

In 1941 many ornamental flowering plants grown in the canyons of the Montara Mountains, where the cut-flower trade is an important industry, were affected with aster yellows. Five acres of strawflowers were destroyed by his disease. Annual phlox manifested both breaking in the petals and symptoms of aster yellows.

Lots of 20 previously noninfective, short-wing and long-wing aster leaf hoppers, *Macrosteles divisus* (Uhl.), after feeding for a few days on diseased phlox removed from the field, were transferred and kept on healthy asters that developed typical symptoms of aster yellows. It is evident from this experiment that phlox was naturally infected with aster yellows.

Attempts were made to infect annual phlox, grown from seed, with the California aster-yellows virus, which is not transmissible to any host plant by means of juice inoculation at room temperature; hence, infective aster

[3] Jong, H. G., Bungenberg de, and J. L. Hartcamp. On the formation of hyaline vesicles at the surface of *Paramecium caudatum.* Protoplasma. 31: 550–587. 1938.

[4] See footnote 1.

[5] Reed, H. S., and Jean Dufrenoy. Pyridoxin and coacervates in plant cells. Science 96: 470. 1942.

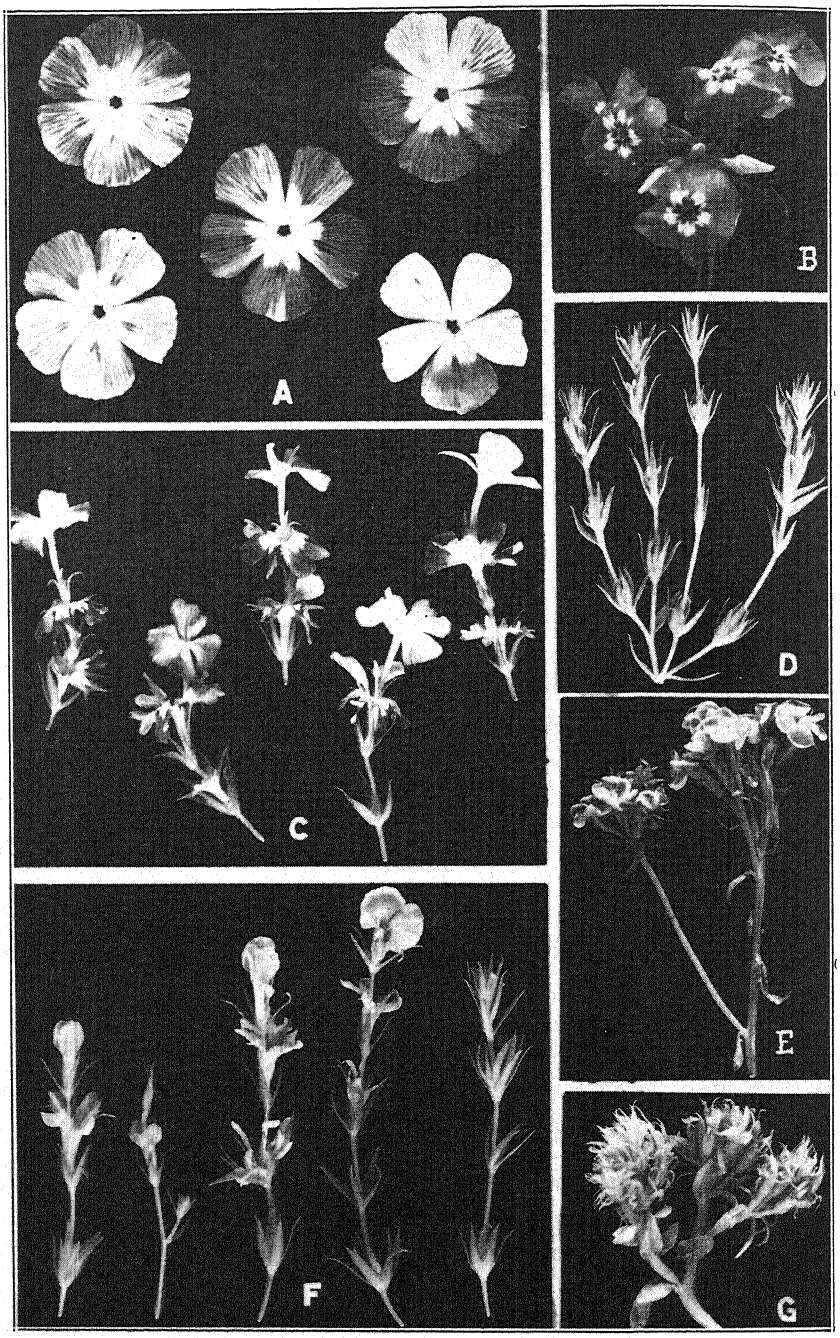



FIG. 1. Symptoms of California aster yellows on annual phlox (*Phlox drummondii*). A. Breaking on color of petals. B. Cluster of flowers from healthy check, or control plant. C. Proliferation of flowers showing breaking in color of petals. D. Proliferation and virescence or greening of flowers, the petals being reduced to leafy structures resembling sepals. E. Virescence of petals. F. Proliferation of flowers with green leafy petals on lower flowers and dwarfed apical flowers with one or more petals absent. G. Cluster of green flowers with no proliferation.

leaf hoppers were used. Healthy phlox plants were exposed 3 weeks or longer to 20 infective male short-wing aster leaf hoppers that had completed the nymphal stages on diseased celery plants or 20 infective male long-wing aster leaf hoppers that had completed the nymphal stages on aster-yellows plants. Males were used rather than females to avoid egg deposition. The males were then removed, and the inoculated plants and the check or control plants were put in an insect-proof cage until the blossoming period. The aster-yellows virus induced breaking in the petals of phlox (Fig. 1, A). The virus was recovered by previously noninfective short-wing and long-wing aster leaf hoppers and transferred to healthy aster and celery plants, respectively.

Breaking in color of the petals consists of white veinbanding, sometimes one or more petals show breaking and the remainder are white with a few streaks of the normal color of the flower (Fig. 1, A). Frequently, virescence or greening of the petals occurs (Fig. 1, E) without breaking. Proliferation of the flowers occurs, successive flowers developing from the ovaries. Breaking often is evident on successive proliferated flowers (Fig. 1, C). In the advanced stage of the disease proliferation and virescence of the flowers are common on infected plants, the petals being reduced to green leafy structures resembling sepals (Fig. 1, D), sometimes, with a dwarfed apical flower with one or more petals absent (Fig. 1, F). Frequently, clusters of green flowers develop with no proliferation of flowers (Fig. 1, G).

It is interesting to note, that this is the first case of a leaf-hopper-transmitted virus inducing breaking in color of flowers.—HENRY H. P. SEVERIN, California Agricultural Experiment Station, Berkeley, California.

*An Unusual Bean Disease.*[1]—An apparently new disease of beans has been observed in field and garden beans grown for seed in southern Idaho. The disease is characterized by a pronounced reddish discoloration of the nodes, which usually is the first indication of its presence. In the later stages the leaves become malformed and the veins are of a decided reddish color (Fig. 1, A). Reddish colored sunken lesions are usually, though not always present on the pods of plants having red nodes and veins (Fig. 1, B).

The disease does not kill the plant, but causes dwarfing, a reduction in the number of pods produced, and premature ripening. When seeds are removed from ripe diseased pods similar to those shown in figure 1, B, they are often severely marked with target-like spots or concentric lines on the seed coats (Fig. 1, C). The seeds from such pods are usually small and often shrivelled.

The disease is more severe in some varieties than in others. The variety Bountiful is particularly susceptible. Great Northern and Red Mexican field beans are also very susceptible, although the disease does not mark the

[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 217.

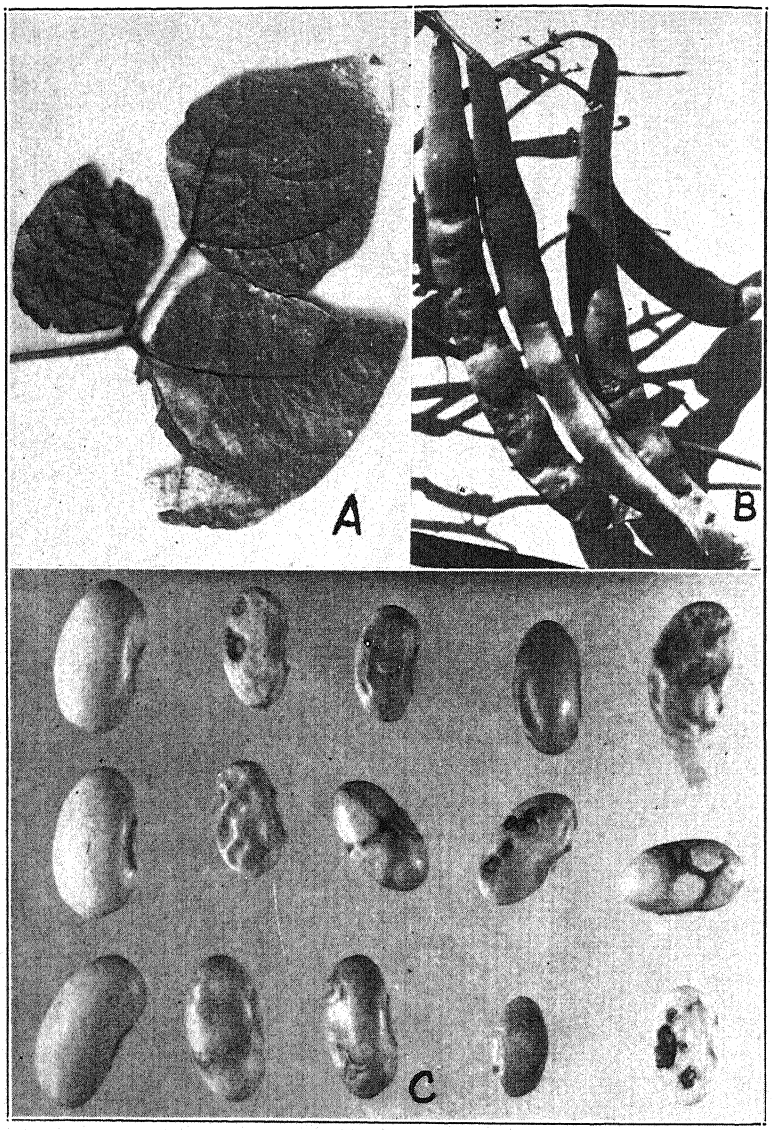



FIG. 1. A. Reddish discoloration of the veins. B. Pods with severe lesions. C. Seeds from infected pods, similar to those shown in B.

seed so severely. The disease has been found to be more prevalent along the edges of bean fields.

Preliminary evidence indicates that the disease is caused by a virus, and appears to be closely related to bean virus 2, or yellow bean mosaic virus.

Affected seeds similar to those shown in figure 1, C, were planted in pots in the greenhouse. Most of the seeds germinated. Plants from 48 seeds were grown to maturity and no sign of the disease was visible on any of the

plants. This would indicate that the disease is not seed borne.—W. J. VIRGIN, Idaho Agricultural Experiment Station, Miscow, Idaho.

*A Leaf Disease of Kentucky Bluegrass.*[1]—In cool, wet periods, particularly in the spring, whole fields and lawns of Kentucky bluegrass, *Poa pratensis,* in central Kentucky may take on a yellowish-brown cast as a result of a leaf disease. This is usually at a time when growing conditions should be about ideal. The same disease also may appear in the fall when it may again cause extensive injury to the foliage. Injury appears to occur first on the leaf sheath, and may spread through the sheath to young leaves or tissues within, eventually killing all leaves of a shoot. The blades of leaves affected on the sheath often begin to yellow at the tips, as well as the base, and finally turn completely yellow and die. Under somewhat drier conditions small dark-brown spots may be seen on leaf blades and sheaths. During the summer new infections are rare. The disease rarely kills plants, but may cause nearly complete defoliation.

In 1940 and since that time, isolations from affected tissue have fairly consistently yielded an organism that J. R. Hardison has recently identified as *Septoria oudemansii* Sacc. Pycnidia of this fungus are commonly found on dead leaf blades. They contain spores that appear to be non-septate, or occasionally 1- or 2-septate, hyaline, and measure about $2.5 \times 14.5$ μ. In culture on potato-dextrose agar at 15° C. black stroma-like bodies were produced in which the pycnidia were immersed. On sterilized bluegrass leaves pycnidia were produced in abundance at 25° C. Sections of infected leaf tissue stained with cotton-blue showed the fungus to be intercellular.

*Septoria oudemansii* has been reported on *Poa pratensis* as a leaf spot in Michigan by Hardison,[2] in Oregon by Sprague,[3] and in Western United States by Fischer *et al.*;[4] but the importance of this fungus as a cause of a serious disease of bluegrass seems not to have been recognized heretofore.—ROBERT B. GRIFFITH, Kentucky Agricultural Experiment Station, Lexington, Ky.

*Powdery Mildew on Ribbon-bush (Homalocladium platycladum Bailey).*—The attention of the writer was recently called to a powdery mildew affecting a plant of the ribbon-bush (*Homalocladium platycladum* Bailey), a native of the Solomon Islands. The plant, a member of the Polygonaceae, is unusual in possessing flattened, strap-like stems (phyllodia), little thicker than the leaves. The leaves are borne singly at the nodes, with their flat surfaces in the same plane as the stem (Fig. 1). Mildew infection was heavy on the leaves, causing yellowing and distortion, but was absent from the stem

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Hardison, J. R. Grass diseases in Michigan in 1941. Plant Dis. Rptr. 26: 67–75, 1942.

[3] Sprague, Roderick. A revised check list of parasitic fungi on cereals and other grasses in Oregon. Plant Dis. Rptr., Supplement 134, 1942.

[4] Fischer, George W., Roderick Sprague, Howard W. Johnson, and John R. Hardison. Host and pathogen indices to the diseases observed on grasses in certain western states during 1941. Plant Dis. Rptr., Supplement 137, 1942.

tissues. Perithecia were abundant and proved to be those of a species of *Erysiphe*, probably *E. polygoni*. Mildew has been severe on the foliage of this plant each winter since it was obtained, some 4 or 5 years ago; but it is

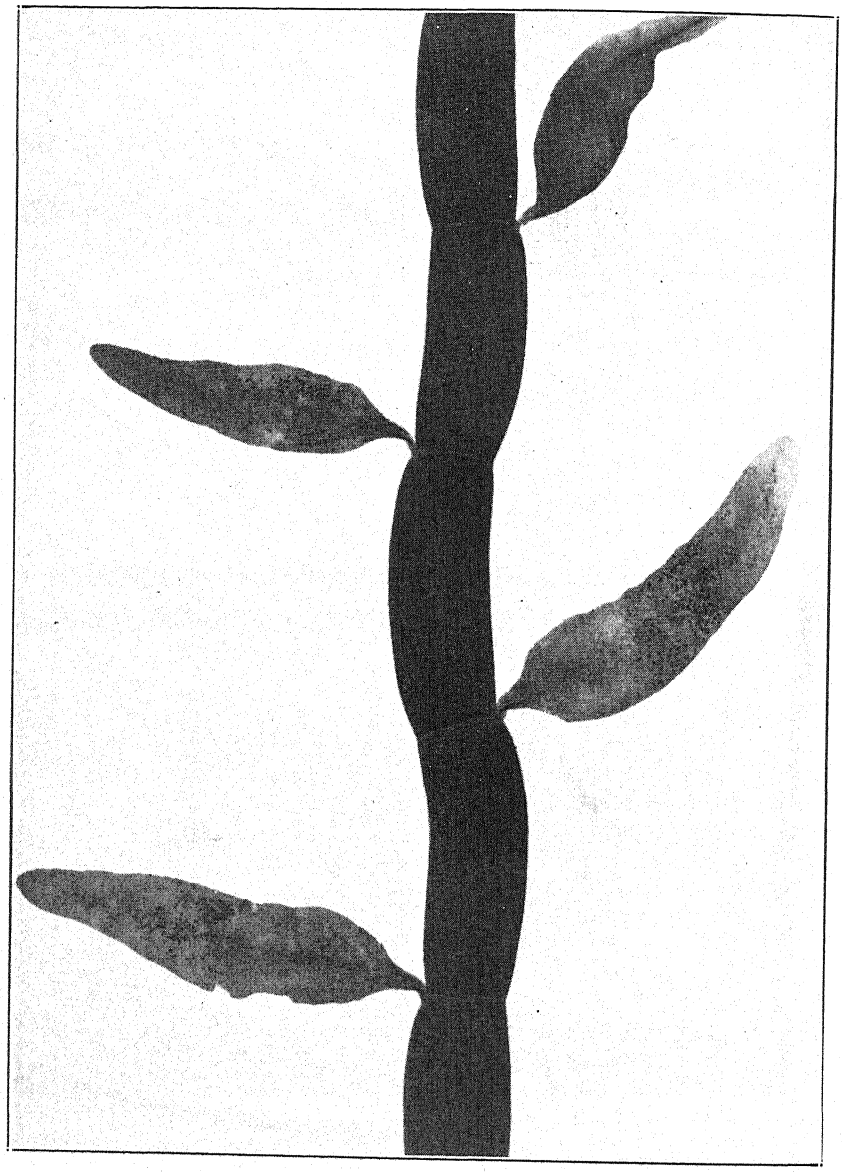


FIG. 1. *Erysiphe* sp. on *Homalocladium platycladum*. Note perithecia on true leaves and absence of infection from the phyllode or stem tissues.

not known whether the mildew was brought in with the plant or came over subsequently from some native host. The writer has been unable to find a previous report of powdery mildew on *H. platycladum*.—A. W. DIMOCK, Cornell University, Ithaca, New York.

## REPORT OF THE WAR COMMITTEE

Reports of subcommittees indicate good progress all along the line. The regional committees have done an excellent job in compiling and publishing in mimeograph form essential facts about the prevalence and control of diseases of major importance in their respective areas. These are being put to good use in extension work throughout the country.

A development of outstanding significance is the recent announcement of the emergency expansion of the Plant Disease Survey in which 24 full-time plant pathologists will be utilized. This should be a potent factor in safeguarding our food supply. It gives us an opportunity to prove the value of the Survey, but it also places a real responsibility upon us to see that this emergency survey is effectively carried out. This is a responsibility, not only of Dr. Edson and his staff, but of each and every member of our Society.

The fungicide subcommittee is again sponsoring cooperative studies on substitute fungicides. One of the most outstanding results of these studies so far is the promising value of a number of organic seed protectants. These appear to give good results on practically all vegetable seeds, and seem to have a wide latitude in dosage without seed injury. Such fungicides should overcome many of the difficulties in the way of pretreatment of packet seeds. Dr. George L. McNew has been assigned the task of investigating the possibility of getting seed dealers to pretreat more of their packet seeds.

The executive committee has spent considerable time on the manpower situation. After a number of conferences the War Committee of the Economic Entomologists joined with this committee in requesting that a national committee on Plant Pathologists and Economic Entomologists be established to advise the National Roster and the Selective Service on matters pertaining to deferment and to more effective use of trained personnel in these fields. Our suggestions were favorably received. The resolution prepared by the War Committee together with data obtained from the survey made by Dr. Keitt have been submitted as directed. At the request of the National Roster, the committee is compiling data on plant pathologists in the armed services for their use in obtaining more efficient use of highly trained personnel already in service.

A reply has been received from Dr. Noble Clark stating the Directors' policy relating to regional meetings. A copy of this letter has been sent to contact men for their guidance in arranging future regional meetings. The essential requirement is that the meeting be sponsored by a Director or the Chief of a Federal Bureau.

Plans are under way for a meeting of the Upper Mississippi Valley Regional Committee in August, at Lafayette, Indiana. It is tentatively

planned to have a meeting of the War Committee in conjunction with this regional meeting.

The Committee has acted favorably upon the suggestion that a subcommittee on Market Pathology be created. Dr. C. O. Bratley has been requested to act as chairman. Other members of the committee will be announced later.

Dr. Stakman has finally returned from his mission to Mexico and has resumed the committee chairmanship.

Respectfully submitted,
J. G. LEACH, Acting Chairman
July 1, 1943

# A HERITABLE ABNORMALITY IN THE GERMINATION OF CHLAMYDOSPORES OF USTILAGO ZEAE[1]

ST. JOHN P. CHILTON

(Accepted for publication January 12, 1943)

## INTRODUCTION

In the course of studies on the genetics of *Ustilago zeae* (Beckm.) Unger, chlamydospores from certain crosses between haploid cultures were found to germinate abnormally. Certain monosporidial cultures, capable of producing galls and chlamydospores when inoculated singly into corn plants, and, therefore, designated as solopathogenic lines, were found among the progeny of these abnormally germinating chlamydospores. A brief report of these studies was made previously (1), and this paper gives a more detailed account of the results obtained.

## REVIEW OF LITERATURE

The general literature concerning the genetics and cytology of *Ustilago zeae* has been reviewed (2, 3, 9, 17), and only certain details need be repeated here. Abnormal germination of smut chlamydospores has been reported by Christensen (3) in one cross in *U. zeae*, by Goldschmidt (7) in *U. violacea*, by Hanna and Popp (10) and Holton (11) in interspecific crosses between *U. avenae* and *U. levis*, by Rodenhiser (14) in crosses between *Sphacelotheca sorghi* and *S. cruenta*, by Laskaris (13) in *S. sorghi*, and by Kienholz and Heald (12) in *Tilletia tritici*. Christensen (3) and Holton (11) suggested that a lethal factor or series of factors might account for the peculiarities in germination that they observed. Laskaris (13) showed that some type of segregation occurred for normal and abnormal germination. Goldschmidt (7) interpreted his results as indicating cytoplasmic inheritance.

Solopathogenic lines in *U. zeae* have been reported by several investigators (1, 2, 3, 5, 18). Christensen (3) found that such lines were indistinguishable in culture from haploid ones, were not dicaryotic, remained solopathogenic for varying lengths of time in culture, and, on losing solopathogenicity, changed in cultural characters also. Sporidia from chlamydospores produced by these lines were generally haploid, although certain chlamydospores again produced solopathogenic lines.

## MATERIALS AND METHODS

The source and parentage of the cultures of *Ustilago zeae* used in the present studies are given in table 1. All cultures, except 15 from cross 50, were derived from single sporidia isolated with a glass needle in a micro-

[1] Summary of a thesis presented in partial fulfillment of the requirements for the degree Doctor of Philosophy, granted by the University of Minnesota, July, 1938.

Paper No. 2055 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

Assistance in the preparation of these materials was furnished by the personnel of Work Projects Administration, Official Project No. 265-1-71-236, Subproject No. 491.

manipulator, using the technique of Dickinson (4) and Hanna (8). The 15 cultures from cross 50 grew from hyphal tips cut from the promycelia.

TABLE 1.—*Origin of cultures used, their source, parentage, and type of isolation*

| Cross number or identification letters and numbers | Parentage | Type of isolation | Where obtained |
|---|---|---|---|
| 10 | A2 by A3 | Complete sets[a] | E. C. Stakman |
| 11 | 10A2 × 10H3 | " " | " |
| 12 | 10K4 × 10G4 | " " | " |
| 14 | 10K2–1 × 10I1 | " " | " |
| 15 | 10K4 × 10F2 | " " | " |
| 16 | 10A4 × 10C1 | " " | " |
| 17 | 10K2–1 × 10I1 | " " | " |
| 18 | 10K2 × 10I1 | " " | " |
| 21 | 16B2 × 16B3 | " " | " |
| 22 | 14G3 × 14I3 | " " | " |
| 23 | 15E2 × 15E4 | " " | " |
| 25 | 14B1 × 14B3 | " " | " |
| 26 | 15E3 × 15E4 | " " | " |
| 33 | 22B2 × 22B4 | " " | " |
| 40 | 10A4 × 15C2 | Random[b] | Writer |
| 41 | 40a × 40i | " | " |
| 42 | 10A4 × 15C1 | " | " |
| 44 | 10A4 × U.F. A3 | " | " |
| 45 | U.F. A1 × A4 | " | " |
| 46 | 16B2 × U.F. A4 | " | " |
| 47 | Solopathogenic line 40n–1 | Complete sets | " |
| 48 | 21H1 × U.F. A4 | " " | " |
| 50 | 10K2–1 × 18H1 | Complete sets + 15 Hyphal tip cultures | M. F. Kernkamp |
| 51 | Solopathogenic line 40n | Complete sets | Writer |
| 53 | 46B3 × U.F. A4 | " " | " |
| 56 | 10K4 × 40i | Random | " |
| 100 | 10K2–1 × 14B3–3 | Complete sets | E. C. Stakman |
| 101 | 100H4 × 202A4 | " " | " |
| 102 | 100C4 × 202A4 | " " | " |
| 202 | 22B4 × 33A2 | " " | " |
| 300 | 14J4 × 26G1–2 | " " | " |
| 301 | 17B4 × 26G1–2 | " " | " |
| U.F. | Chlamydospores from University Farm, St. Paul, Minn. | " " | Writer |
| Fargo | Chlamydospores from Fargo, N. D. | " " | " |

[a] Cultures from all 4 sporidia from a promycelium were secured wherever possible.
[b] No attempt made to secure cultures from all primary sporidia from a promycelium.

The system used to identify the various cultures was as follows: An arbitrary number was assigned to each cross and to chlamydospores produced by solopathogenic lines. Letters or words were used to designate the source of the material when isolations were made from material collected in the field. Where sporidia were isolated from a promycelium and the position of each was known, the chlamydospore was identified by a capital letter and the sporidia were numbered from 1 to 4, progressing from the distal to the proximal cell. For example, 16A1 designates a line obtained from the sporidium isolated from the distal cell of the promycelium of chlamydospore

A from Cross 16. If sporidia were isolated at random from promycelia, the cultures were designated by one or more small letters placed after the cross number (40a or 40aa). Genetic variants (mutants) are indicated by a number placed after the culture designation and separated by a dash (16A1–1 or 40a–1).

In the experiments on sex and pathogenicity, Northwestern Dent and Picaninny corn plants were grown in sterilized soil in 4- or 6-inch pots from seed treated with an organic dust. The inoculation technique described by Stakman and Christensen (16) was followed. Solopathogenicity tests were made by inoculating plants with monosporidial cultures. Except where stated, a minimum of two tests, with 10 to 20 plants in each test, was made with each cross or solopathogenic line; and in many cases 8 to 10 tests were made.

Cultures were grown on 50 cc. or 30 cc. of potato-dextrose agar (10 g. dextrose, 15 g. agar, and infusion of 300 g. of potatoes to a liter of medium) in 250-cc. or 125-cc. Erlenmeyer flasks. All the medium used in each experiment was made at the same time, and the flasks were sterilized in the autoclave in groups of 100, the sterilization time and pressure being alike for the groups. Duplicate flasks were inoculated with each culture and were incubated at room temperature for 5 to 8 weeks, after which notes were taken.

The studies on chlamydospore germination were made at room temperature on thin films of potato-dextrose agar on sterile cover slips inverted on sterile Van Tieghem cells in Petri dishes. Moistened filter paper was used in the bottom of the dishes. Special media are described in connection with the experiments in which they were used. Chlamydospores were spread on the agar films with a glass needle in a micromanipulator, preparatory to isolating single sporidia.

## GERMINATION OF CHLAMYDOSPORES

Chlamydospores that germinate normally produce a promycelium with 3 or 4 cells on each of which sporidia are usually formed (Fig. 1, A). The chlamydospore may act as the fourth cell in some cases, producing one or more sporidia.

The chlamydospores that germinated abnormally produced promycelia in about the same length of time as those that germinated normally, but some of the promycelia collapsed and the contents exuded before septa were formed (Fig. 1, B). The word lysis will be used hereafter to designate this disintegration. A round body suggestive of an abortive sporidium was often produced on the side of the promycelium (Fig. 1, D). Lysis occurred if the chlamydospores were dusted on agar films, indicating it was not due to rough handling. Promycelia that did not lyse generally produced viable sporidia that grew into colonies, although in some cases the isolated sporidia budded 2 or 3 times and then died. This limited budding was seen occasionally also in otherwise normally germinating collections of chlamydospores. Many of the promycelia producing sporidia were gnarled and twisted (Fig. 1, C), others were made up of large, irregular cells, and it was difficult to find pro-

mycelia with 4 cells in any of the abnormal material studied. Long hyphal branches sometimes were formed directly from the chlamydospores or from the promycelia. A few germination types superficially similar to those found in the Tilletiaceae were seen, a whorl of sporidia or sporidia and

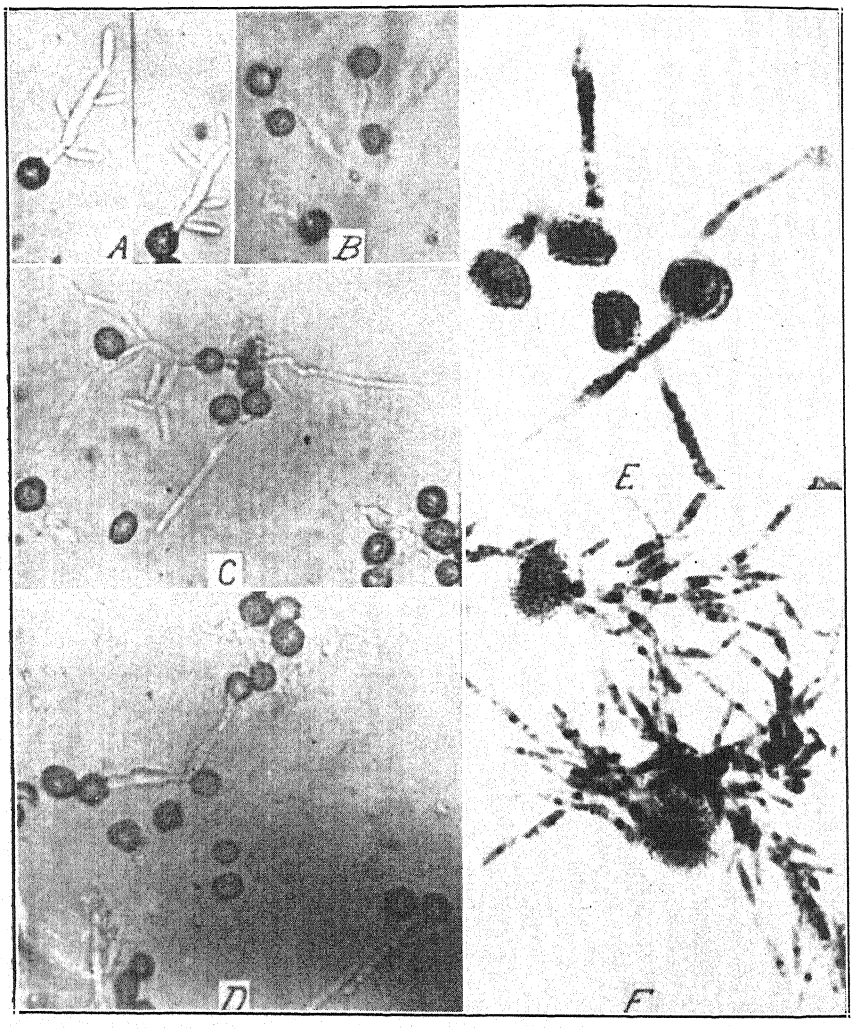



FIG. 1. Photomicrographs of abnormally and normally germinating chlamydospores. A. Normally germinating chlamydospores. B–D. Abnormally germinating chlamydospores. E. Abnormally germinating chlamydospores stained with hematoxylin. F. Normally germinating chlamydospores stained with hematoxylin.

hyphal branches being formed at the tip of a one-cell promycelium. Many chlamydospores produced 2 irregular promycelia.

The proportion of promycelia that lysed varied from 76 to 95 per cent in the various crosses studied. In one case a consistent 15 per cent difference in the number of promycelia that lysed was found between two col-

lections of chlamydospores produced by inoculations made at different times with the same two compatible lines.

When enough promycelia were examined from crosses that produced otherwise normally germinating chlamydospores, a few promycelia were seen to lyse in nearly every case, the percentage varying from 8.3 to 0.3 per cent. This occurred, even when the chlamydospores were dusted on coverslips to eliminate the possibility of injury.

Normally (Fig. 1, F) and abnormally germinating chlamydospores (Fig. 1, E) on the same films of agar were stained with hematoxylin in accordance with the technique described by Hanna (9). The cytoplasm in many of the abnormal promycelia stained much darker than that of the normal ones on the same cover slip; and there often was a concentration of cytoplasm in various parts of a promycelium (Fig. 1, E). Nuclei were observed in chlamydospores prior to germination, at the beginning of germination, in the promycelia producing sporidia, and in the sporidia themselves.

#### INFLUENCE OF ENVIRONMENT ON ABNORMAL GERMINATION

Several collections of chlamydospores that had germinated normally and abnormally, respectively, in previous tests on potato-dextrose agar were tested in sterile distilled water; in potato broth with 1 per cent and 20 per cent dextrose; in 2 per cent water agar, with and without 0.1 per cent NaCl; and on corn-meal agar, malt agar, potato-dextrose agar, and on potato-dextrose agar to which 1, 5, and 10 drops of N/10 NaOH, and 1, 2, 3, and 5 drops of commercial lactic acid, respectively, had been added to 10-cc. portions. None of these media was responsible for the peculiarities of germination of the chlamydospores of the various collections. Lysis occurred in the abnormally germinating chlamydospores at 15, 20, 25, and 30 degrees C.

### Attempts to Induce Abnormal Germination

On the assumption that lysis might be due to a virus or some similar substance, a few chlamydospores were placed on a thin film of agar and left until promycelia were produced and lysed. Chlamydospores from a normally germinating collection were then added but there was no lysis or other observable abnormality, even in promycelia in direct contact with the lysed ones. This was repeated many times, with the same negative results.

Small pellets of agar on which 5 to 6 chlamydospores had germinated and the promycelia had lysed were added to compatible lines growing in potato dextrose broth, and these were left standing for approximately a week. The lines were then mixed, inoculated into corn plants, and the chlamydospores that were produced germinated normally.

These results indicate that the abnormality was not due to an infectious agent, or at least was not transmitted by the methods used.

### Segregation for Abnormal and Normal Germination

The chlamydospores produced by the original cross 10, from which most of the material used in these studies was obtained, germinated abnormally.

Haploid lines isolated from this cross were mated in various compatible combinations, and some of the resultant crosses produced chlamydospores that germinated abnormally, which others produced chlamydospores that

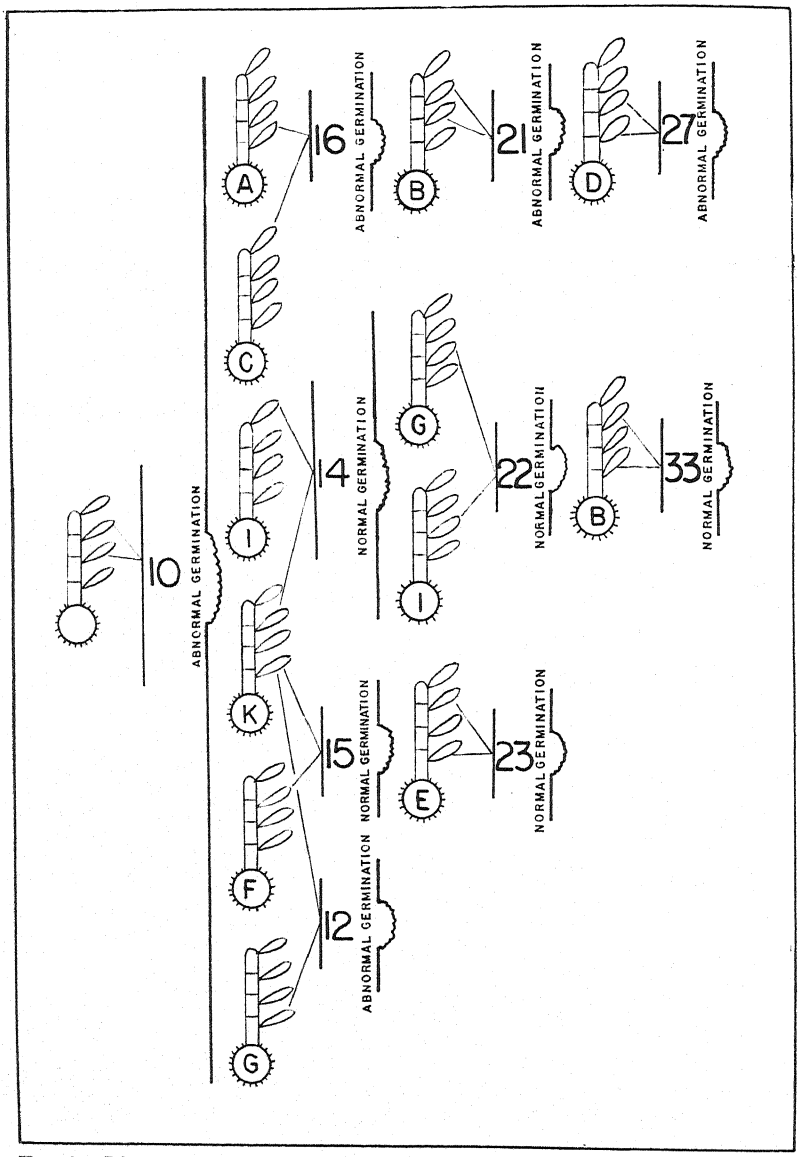



FIG. 2. Diagrammatic presentation of segregation for normal and abnormal germination of chlamydospores of *Ustilago zeae*.

germinated normally. Monosporidial isolates from these crosses were intercrossed in turn. The results, presented diagrammatically in figure 2, show that chlamydospore lines were obtained from the original cross in which normal germination occurred for 3 and 2 successive chlamydospore gener-

ations, respectively, and that other chlamydospore lines were obtained in which abnormal germination occurred for 3 and 1 successive chlamydospore generations, respectively. A segregation of some type occurred for abnormal and normal germination of chlamydospores.

From 5 of the crosses made within the progeny of the original cross 10 (Fig. 2) there were 13 haploid lines which produced abnormally germinat-

TABLE 2.—*Results of crosses*[a] *made to determine whether there was segregation for normal and abnormal germination*[b] *of chlamydospores*

| Lines crossed | Fargo B1 | Fargo B2 | 10K2 | 10K4 | 10H3 | 10C1 | 10I1 | U.F. A4 | 15C1 | 15C2–1 | 16B2 | 21D3 | 21H1 | 40a |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10A2 | | | | | A | | | | | | | | | |
| 10A4 | | | | | | A | | | | A | | | | |
| 10C1 | N[b] | | | | | | | | | | | | | |
| 10C2 | N | | | | | | | | | | | | | |
| 10F1 | | N | | | | | | | | | | | | |
| 10F2 | N | | | N | | | | | | | | | | |
| 10G4 | | | | A | | | | | | | | | | |
| 10H4 | N | | | | | | | | | | | | | |
| 10I1 | | | N | | | | | | | | | | | |
| 10I3 | N | | | | | | | | | | | | | |
| 10K2 | | N | | | | | N | | | | | | | |
| 10K4 | | N | | | | | N | | | | | | | |
| 12D4 | | A | | | | | | | | | | | | |
| 12E1 | | N | | | | | | | | | | | | |
| 16A1 | | | | | | | | N | | | | | | |
| 16A4 | | N | | | | | | N | | | | | | |
| 16B2 | | | | | | | | N | | | | | | |
| 16B3 | A[b] | | | | | | | | | | A | | | |
| 21A4 | | N | | | | | | | | | | | | |
| 21C3 | | N | | | | | | | | | | | | |
| 21D3 | N | | | | | | | | | | | | | |
| 21D4 | | | | | | | | | | | | A | | |
| 21F2 | N | | | | | | | | | | | | | |
| 21H1 | | N | | | | | | N | | | | | | |
| 21H2 | | N | | | | | | N | | | | | | |
| 21H3 | | A | | | | | | A | | | | | | |
| 21H4 | N | | | | | | | | | | | | N | |
| 21I4 | N | | | | | | | | | | | | | |
| 40a | | N | | | | | | | | | | | | |
| 40f | | | | | | | | | | A | | | | |
| 40i | | | | A | | | | | | A | | | | A |
| 40q | | | | | | | | | A | A | | | | |
| 40r–1 | | | | | | | | | A | A | | | | |
| 40dd | | | | | | | | | | A | | | | |
| 40B3 | | A | | | | | | | | | | | | |

[a] Crosses (as diagrammed in Fig. 2): 10 = A2 × A3
12 = 10K4 × 10G4
16 = 10A4 × 10C1
21 = 16B2 × 16B3
40 = 10A4 × 15C2

[b] N indicates normal germination; A indicates abnormal germination.

ing chlamydospores and 23 lines which produced normally germinating chlamydospores when mated with various tester lines (Table 2). The tester lines all produced normally germinating chlamydospores when mated among themselves.

From each of two crosses that produced normally germinating chlamydospores a haploid line was secured which, when crossed with tester lines, produced chlamydospores that germinated abnormally.

### OCCURRENCE OF SOLOPATHOGENIC LINES

Sporidia were isolated from collections of normally and abnormally germinating chlamydospores produced by various crosses, and the cultures obtained were tested for solopathogenicity by inoculation tests. It may be seen from the data in table 3 that 31 or 19.6 per cent of the 158 abnormally

TABLE 3.—*Occurrence of solopathogenic lines among progeny of 29 crosses*

| Cross number | Parentage | Occurrence of abnormal germination | No. chlamydospores from which one or more isolates were tested | Number isolates tested | No. chlamydospores from which solopathogenic lines were isolated | No. of solopathogenic lines |
|---|---|---|---|---|---|---|
| 10 | A2 × A3 | + | 11 | 39 | 3 | 11 |
| 11 | 10A2 × 10H3 | + | 8 | 24 | 0 | 0 |
| 16 | 10A4 × 10C1 | + | 6 | 16 | 0 | 0 |
| 42 | 10A4 × 15C1 | + | 24 | 24 | 3 | 3 |
| 40 | 10A4 × 15C2 | + | 39 | 39 | 10 | 10 |
| 44 | 10A4 × U.F. A3 | + | 6 | 20 | 1 | 4 |
| 12 | 10G4 × 10K4 | + | 7 | 20 | 2 | 2 |
| 21 | 16B2 × 16B3 | + | 6 | 16 | 0 | 0 |
| 41 | 40a × 40i | + | 31 | 40[a] | 6 | 6 |
| 56 | 40i × 10K4 | + | 1 | 1 | 1 | 1 |
| 45 | U.F. A1 × A4 | + | 19 | 19 | 5 | 5 |
| | Total ..... | ..... | 158 | 258 | 31 | 42 |
| 14 and 17 | 10K2–1 × 10I1 | – | 20 | 54 | 0 | 0 |
| 18 | 10K2 × 10I1 | – | 8 | 24 | 0 | 0 |
| 50 | 10K2–1 × 18H1 | – | 22 | 39 | 0 | 0 |
| 100 | 10K2–1 × 14B3–3 | – | 4 | 8 | 0 | 0 |
| 15 | 10K4 × 10F2 | – | 5 | 22 | 0 | 0 |
| 25 | 14B1 × 14B3 | – | 5 | 13 | 0 | 0 |
| 22 | 14G3 × 14I3 | – | 4 | 16 | 0 | 0 |
| 23 | 15E2 × 15E4 | – | 5 | 20 | 1 | 1[b] |
| 26 | 15E3 × 15E4 | – | 7 | 10 | 0 | 0 |
| 46 | 16B2 × U.F. A4 | – | 18 | 39 | 0 | 0 |
| 300 | 14J4 × 26G1–2 | – | 7 | 13 | 0 | 0 |
| 301 | 17B4 × 26G1–2 | – | 7 | 9 | 0 | 0 |
| 48 | 21H1 × U.F. A4 | – | 11 | 23 | 0 | 0 |
| 33 | 22B2 × 22B4 | – | 5 | 19 | 0 | 0 |
| 202 | 22B4 × 33A2 | – | 8 | 20 | 0 | 0 |
| 53 | 46B3 × U.F. A4 | – | 4 | 7 | 0 | 0 |
| 101 | 100H4 × 202A4 | – | 1 | 2 | 0 | 0 |
| 102 | 100C4 × 202A4 | – | 4 | 4 | 0 | 0 |
| | Total ..... | ..... | 145 | 342 | 1 | 1 |

[a] Some of the haploid lines from this cross were tested alone only once. The solopathogenic lines, however, were tested several times.

[b] This line's cultural characters changed and solopathogenicity was lost on transfer of stock culture.

germinating chlamydospores from which one or more cultures were isolated and tested, gave rise to one or more solopathogenic lines. There were 42 solopathogenic lines among the 258 monosporidial isolates tested, an average of 16.3 per cent. Three hundred and forty two lines from 145 chlamydospores from 18 crosses producing normally germinating chlamydospores were tested and only one line, or 0.29 per cent, was solopathogenic. This line, however, lost its solopathogenicity on transfer of the stock culture.

Five solopathogenic lines were found among 19 monosporidial isolates from the abnormally germinating chlamydospores produced by cross 45 (U.F. A1 × U.F. A4). This indicates that solopathogenic lines were not restricted to the relatively closely related lines used in these studies, as the chlamydospores from which cultures U.F. A1 and A4 were secured were collected in the experimental plots at University Farm, St. Paul, Minnesota.

## Cultural Characters of Solopathogenic Lines

From 5 crosses, cultures of lines that had been proved by inoculation to be solopathogenic were compared with each other and their parents. The results (Table 4) show that solopathogenic lines from a given cross may be

TABLE 4.—*Comparison of cultural characters of solopathogenic lines with each other and with parental types*

| Cross number | Parental lines | No. of solopathogenic lines compared | Parent resembled | No. of cultural types |
|---|---|---|---|---|
| 40 | 10A4 × 15C2 | 10 | 10A4 | 7 |
| 41 | 40a × 40i | 6 | 40i | 3 |
| 42 | 10A4 × 15C1 | 3 | 10A4 | 2 |
| 44 | 10A4 × U.F. A3 | 4 | Both | 1 |
| 45 | U.F. A1 × U.F. A4 | 5 | Neither | 5 |

the same or different, culturally. No character was found by which these lines could be distinguished culturally from haploid ones. These results are in accord with those of Christensen (2, 3).

## Stability of Solopathogenic Lines

Lines were tested for solopathogenicity at various intervals, and the results (Table 5) show that some lines remained solopathogenic after 4 years

TABLE 5.—*Results of testing 31 originally solopathogenic lines after varying lengths of time in culture*

| Cross from which lines were obtained | Age in months when last tested | No. of lines tested | Number solopathogenic | Number not solopathogenic |
|---|---|---|---|---|
| 10 | 48 | 8 | 7 | 1 |
| 12 | 36 | 1 | 1 | 0 |
| 40 | 13 | 9 | 9 | 0 |
| 40 | 9 | 1 | 1 | 0 |
| 41 | 11 | 5 | 5 | 0 |
| 42 | 8 | 4 | 4 | 0 |
| 44 | 5 | 4 | 1 | 3 |

in culture, while others lost this character after a few months. Where solopathogenicity was lost, the cultural characters changed also.

Single sporidia were isolated from solopathogenic lines at various times and the cultures obtained were tested by inoculation into corn plants. The results (Table 6) show that monosporidial isolates from such lines may or may not be solopathogenic.

TABLE 6.—*Results of inoculations with sub-monosporidial isolates from solopathogenic lines*

| Solopathogenic lines | Number sub-monosporidial isolates tested | Number solopathogenic | Number not solopathogenic |
|---|---|---|---|
| 10J4 | 7 | 5 | 2 |
| 40n | 2 | 1 | 1 |
| 40m | 1 | 1 | 0 |
| 41a–1 | 1 | 1 | 0 |
| 41g | 1 | 1 | 0 |
| 41v | 1 | 1 | 0 |
| 41k | 1 | 1 | 0 |
| 41gg | 1 | 1 | 0 |
| 41kk | 1 | 1 | 0 |
| 42r | 1 | 1 | 0 |
| 42u | 1 | 1 | 0 |
| 44A1 | 2 | 0 | 2 |
| 44A2 | 1 | 0 | 1 |
| 44A3 | 1 | 0 | 1 |
| 44A4 | 1 | 0 | 1 |
| 45g | 1 | 1 | 0 |
| 45l | 1 | 0 | 1 |
| 45n | 1 | 1 | 0 |
| 45q | 1 | 1 | 0 |

Monosporidial isolates were made from variants arising in solopathogenic lines and were inoculated into corn plants. Of 14 monosporidial isolates made from 5 variants, all were solopathogenic. These results agree also with those obtained by Christensen (3).

The non-solopathogenic monosporidial isolate from the solopathogenic line 44A3 (Table 6) was inoculated into corn plants in combination with each of 2 haploid compatible lines. It formed galls and chlamydospores in combination with one line and not with the other. This test was not repeated, and too much emphasis should not be placed on it. It suggests, however, that a delayed segregation for compatibility factors may possibly account for loss of solopathogenicity in culture.

### Germination of Chlamydospores Produced by Solopathogenic Lines

In some cases chlamydospores formed by solopathogenic lines produced fewer promycelia that lysed than the chlamydospores from the original cross from which these lines were obtained. To eliminate the possibility that excessive handling of the chlamydospores in spreading them on the agar might cause lysis, chlamydospores produced by 6 solopathogenic lines were placed on thin films of sterile agar and touched lightly with other films of agar to spread the spores. Some of the promycelia still lysed.

Forty-two to 96 per cent of the chlamydospores from 10 solopathogenic lines from 3 crosses produced promycelia that lysed (Table 7). From

TABLE 7.—*Comparison of percentage promycelial lysis in chlamydospores produced by three lethal crosses and by solopathogenic lines from these crosses*

| Source of chlamydospores | No. spores germinated | Percentage of lysed promycelia |
|---|---|---|
| Cross 40 (10A4 × 15C2) | 653 | 85.0 |
| Six solopathogenic lines from Cross 40 | 1853 | 42.1 |
| Cross 44 (10A4 × U.F. A3) | 265 | 80.4 |
| Two solopathogenic lines from Cross 44 | 287 | 47.7 |
| Cross 45 (U.F. A1 × U.F. A4) | 867 | 94.2 |
| Two solopathogenic lines from Cross 45 | 543 | 96.3 |

these data it may be seen that the chlamydospores obtained from the solopathogenic lines from crosses 40 (10A4 × 15C2) and 44 (10A4 × U.F. A3) produced fewer promycelia that lysed than did the chlamydospores from the original crosses. The 2 solopathogenic lines tested from cross 45 (U.F. A1 × U.F. A4) produced chlamydospores in which lysis occurred as often as in the original chlamydospore material from which they were obtained. Chlamydospores from 2 solopathogenic lines from cross 42 (10A4 × 15C1) were tested several times also, although no counts were made; it was found that fewer promycelia disintegrated than in the case of chlamydospores from the original cross.

Among chlamydospores produced by the solopathogenic lines from crosses 40, 42, and 44, no torulose and large-celled promycelia were observed. Most of these lines produced chlamydospores with promycelia that were apparently normal in shape; there was little difficulty in finding 4-celled promycelia with sporidia on each cell. In a few of these lines the chlamydospores formed promycelia with less than 4 cells.

Solopathogenic lines from the same cross differed with respect to the viability of the sporidia isolated. Single sporidia isolated from each of the cells of the promycelia of chlamydospores produced by solopathogenic lines 40n and 40n–1 grew readily, but a rather large number of the sporidia isolated from the promycelia of the chlamydospores produced by 40cc, 40e, and 40e–1 died or budded only once or twice. In some cases, the isolated sporidium produced a hyphal strand, which soon died. Sporidia were isolated from several collections obtained from inoculations made at different times and they behaved in the same way.

Differences in the percentage of promycelial lysis were found among chlamydospores produced by solopathogenic lines from the same cross. For example, 16.5 per cent of the promycelia from line 40e disintegrated, while 49 per cent and 50 per cent of those from lines 40n and 40n–1, respectively, disintegrated.

The germination of chlamydospores produced by 25 solopathogenic lines from 5 different crosses was observed. With the exception of the chlamydospores from the solopathogenic lines obtained from cross 45 (U.F. A1 ×

U.F. A4), the germination was much closer to normal than in the case of the chlamydospores produced by the original cross.

### Segregation and Mutation in Solopathogenic Lines

From cross 40 (10A4 × 15C2) a solopathogenic line was obtained that was almost identical culturally to the 10A4 parent. A variant arose in this line, and a monosporidial isolate was made from it that proved to be solopathogenic. The variant later gave rise in turn to a variant, and a monosporidial isolate from it was also solopathogenic. At the same time it was found that the original 15C2 parent had produced a variant in culture which was still haploid, based on inoculation tests alone and in combination with compatible lines.

Culture 10A4 was brown and smooth on the surface. The culture 15C2 was brown also, but its surface was convoluted and the margin had counterclockwise lines. Culture 15C2–1, the variant from 15C2, was light buff and had a fine white mycelial covering over a rough surface. The variant 40n–1 from the solopathogenic line 40n was brown with a fine white mycelial growth over the surface also.

From the promycelia of 8 chlamydospores produced by the solopathogenic line 40n, 5 sets of 4 sporidia and 3 sets of 3 sporidia were isolated, and the resulting cultures were grown in duplicate flasks. Seven lines were obtained with cultural characters like 10A4 and 4 like 15C2. The other 16 lines were similar to both 10A4 and 15C2. Both the 10A4 and 15C2 types were recovered from the promycelia of 3 of the 8 chlamydospores.

From the chlamydospores produced by the solopathogenic variant 40n–1, 9 sets of 4 sporidia and 5 sets of 3 sporidia were isolated and compared in duplicate flasks. Five of these 51 lines were like 10A4 culturally, 5 resembled 15C2–1 rather closely, and 8 lines had a white overgrowth. Twenty other lines were like 10A4 in color but like 15C2–1 in topography, being rough on the surface. None was like 15C2.

Four of the monosporidial isolates from the chlamydospores produced by 40n and 16 of those obtained from 40n–1 were inoculated into corn plants alone and in various combinations. None was solopathogenic and all formed chlamydospores in certain combinations. These tests, repeated, gave similar results. Among the lines used were those resembling 10A4, 15C2, and 15C2–1 in cultural characteristics.

These results indicate that the solopathogenic line 40n was diploid, as the original parental types were recovered; and segregation occurred for sex or compatibility factors and for cultural characters. They also indicate that the variant from the solopathogenic line was produced as a result of a heritable change, and that rather similar mutations for cultural characters could occur in a genom whether alone or included in a nucleus with another genom.[2]

[2] Unpublished cytological results of E. C. Stakman have shown that the solopathogenic lines used in these studies were not dikaryotic.

### CHLAMYDOSPORE SIZE

The chlamydospores that germinated abnormally were larger than those that germinated normally. Christensen (3) found this to be true also of the abnormally germinating chlamydospores he studied.

One hundred chlamydospores from each of 21 crosses were measured with an ocular screw micrometer, the spores being measured as they lay on a film of agar (Table 8). The data were analyzed by the method given by Fisher

TABLE 8.—*A comparison of average lengths and widths (in microns) of 100 chlamydospores from each of 21 crosses within Ustilago zeae*

| Abnormally germinating chlamydospores | | | Normally germinating chlamydospores | | |
|---|---|---|---|---|---|
| Cross | Length | Width | Cross | Length | Width |
| 10A4 × 10C1 | 10.28 | 9.68 | 16A1 × U.F. A4 | 7.87 | 7.42 |
| 10A4 × 15C2 | 10.48 | 9.84 | 21H1 × U.F. A4 | 8.39 | 8.05 |
| 16A3 × U.F. A1 | 10.57 | 8.06 | 21H2 × U.F. A4 | 8.35 | 7.74 |
| 16B3–1 × Fargo B1 | 11.05 | 9.46 | 21H4 × Fargo B1 | 8.29 | 7.89 |
| 21H3 × U.F. A4 | 10.82 | 9.01 | 21C3 × Fargo B2 | 8.49 | 7.57 |
| 21H3 × Fargo B2 | 10.83 | 9.92 | 22B2 × Fargo B2 | 8.39 | 7.91 |
| 21A1 × U.F. A1 | 9.65 | 9.07 | 46D1 × U.F. A4 | 8.89 | 7.24 |
| 40f × 15C2 | 11.03 | 9.37 | 36E3 × 16B2 | 8.86 | 7.27 |
| 40a × 40i | 10.16 | 9.56 | Fargo B1 × B2 | 8.57 | 8.13 |
| 44B3 × Fargo B2 | 10.72 | 9.80 | U.F. A4 × Fargo B1 | 8.63 | 8.05 |
| U.F. A1 × A4 | 11.69 | 8.14 | | | |

(11), and the "F" value was obtained from the tables of Snedecor (15). The lengths and widths of the abnormally germinating chlamydospores produced by the 11 lethal crosses were 9.7 μ – 11.7 μ by 8.1 μ – 9.9 μ; and 7.9 μ – 8.9 μ by 7.2 μ – 8.1 μ in the case of the normally germinating chlamydospores

TABLE 9.—*Analysis of variance of lengths and widths of chlamydospores of 21 crosses of Ustilago zeae*

| Variation | Degrees of freedom | Sum of squares | Variance | F |
|---|---|---|---|---|
| **Length** | | | | |
| Within abnormally germinating group | 10 | 3,111.51 | 311.15 | 8.04[b] |
| Within normally germinating group | 9 | 1,446.50 | 160.72 | 4.16[a] |
| Between two groups | 1 | 48,097.99 | 48,097.99 | 1,243.48[b] |
| Within crosses | 2079 | 80,409.08 | 38.68 | |
| Total | 2099 | 133,065.08 | | |
| **Width** | | | | |
| Within abnormally germinating group | 10 | 7,416.46 | 741.46 | 40.19[b] |
| Within normally germinating group | 9 | 1.784.44 | 198.27 | 10.74[b] |
| Between two groups | 1 | 22,050.55 | 22,050.55 | 1,194.5[b] |
| Within crosses | 2079 | 38,383.55 | 18.46 | |
| Total | 2099 | 69,635.00 | | |

[a] Value of "F" exceeds 5 per cent point.
[b] Value of "F" greatly exceeds 1 per cent point.

from the 10 non-lethal crosses. The chlamydospores from many of the lethal crosses were rather irregular in outline, and a few were found with distinct apicula.

Chlamydospores produced by 90 crosses among the various lines used in these studies were checked by observation as to size and then were germinated. The chlamydospores of 28 of these crosses were large and germinated abnormally, while those produced by the other 62 crosses were smaller and germinated in the normal way.

The data in table 9 not only indicate that the difference in size is significant between chlamydospores produced by lethal and non-lethal crosses, but also show that there were significant differences among the various collections measured.

Chlamydospores produced by solopathogenic lines were compared with those of chlamydospores from the original crosses from which the solopathogenic lines arose. Chlamydospores produced by two crosses between compatible haploid lines obtained from the chlamydospores produced by the solopathogenic line 40n–1 were measured also. The data (Table 10) show

TABLE 10.—*Comparative widths and lengths of chlamydospores of 2 abnormally germinating crosses, 6 solopathogenic lines, and 2 crosses between segregates from solopathogenic line 40n–1.*

| Source of chlamydospores | Length (in microns) | Width (in microns) |
|---|---|---|
| Cross 40 (10A4 × 15C2) | 10.48 | 9.84 |
| Solopathogenic line 40e | 8.51 | 7.69 |
| Solopathogenic line 40m | 8.28 | 7.60 |
| Solopathogenic line 40n | 8.45 | 7.93 |
| Solopathogenic line 40n–1 | 8.28 | 7.60 |
| 47A1 × A2[a] | 7.95 | 7.76 |
| 47D2 × D3[a] | 8.46 | 7.81 |
| Cross 45 (U.F. A1 × A4) | 11.69 | 8.14 |
| Solopathogenic line 45p | 10.48 | 8.34 |
| Solopathogenic line 45q | 11.71 | 8.44 |

[a] Cross made between 2 segregates obtained by isolating sporidia from chlamydospores produced by solopathogenic line 40n–1.

that the chlamydospores produced by the solopathogenic lines obtained from cross 40 (10A4 × 15C2) were of the same size as those produced by crosses between non-lethal lines, as given in table 8. The chlamydospores produced by the solopathogenic lines from cross 45 (U.F. A1 × U.F. A4) were like those of the parental cross. Those produced by the two crosses between haploid isolates from 40n–1 were more nearly the size of the chlamydospores produced by the solopathogenic line 40n–1 than the ones produced by the original cross 40 (10A4 × 15C2).

As stated previously, the chlamydospores produced by the solopathogenic lines from crosses 40 (10A4 × 15C2) and 44 (10A4 × U.F. A3) were more nearly normal in their germination type than the chlamydospores of the original crosses from which they were obtained. In cross 45 (U.F. A1 × A4), however, the chlamydospores produced by the solopathogenic lines 45p and 45q germinated as abnormally as the original chlamydospores from which

they were obtained. Comparisons of germination type, percentage of lysis, and spore size are given in table 11. There is a correlation between spore

TABLE 11.—*Comparison of chlamydospore size, germination type, and lysis of original crosses, solopathogenic lines, and re-combinations of isolates from solopathogenic line 40n–1.*

| Cross | Size[a] of chlamydospores | Type of germination | Percentage of lysis |
|---|---|---|---|
| 40 (10A4 × 15C2) | Large | Abnormal | 85.0 |
| 6 solopathogenic lines | Normal | Normal | 42.1 |
| 47A1 × A2[b] | " | " | 59.6 |
| 47D2 × D3 | " | " | 29.8 |
| 44 (10A4 × U.F. A3) | Large | Abnormal | 80.4 |
| 2 solopathogenic lines | Normal | Normal | 47.7 |
| 45 (U.F. A1 × A4) | Large | Abnormal | 94.2 |
| 2 solopathogenic lines | " | " | 96.3 |

[a] Size was determined by measurement or was estimated by visual comparison with other measured chlamydospores from similar lines.

[b] Haploid lines isolated from promycelia of chlamydospores produced by the solopathogenic line 40n–1.

size and abnormalities in germination, the solopathogenic lines from crosses 40 (10A4 × 15C2) and 44 (10A4 × U.F. A3) producing the smaller chlamydospores with the more normal germination, while the chlamydospores produced by the solopathogenic lines 45p and 45q were like those of the parental cross 45 (U.F. A1 × A4) with respect to size and abnormal germination.

## DISCUSSION

Preceding experiments show that the abnormal germination of chlamydospores from certain crosses between haploid lines of *Ustilago zeae* is due to heritable factors. Haploid lines that carry the factor or factors for lysis, and diploid solopathogenic lines, can both be propagated indefinitely in culture; hence the operation of the lethal seems to be confined to the time in the life cycle when meiosis occurs. The high percentage of solopathogenic lines among the surviving monosporidial isolates from abnormally germinating chlamydospores indicates that the peculiar germination tends to be associated with a failure of normal disjunction of chromosomes. As the percentage of chlamydospores that lysed differed among chlamydospores produced by different crosses, it would seem that the abnormal germination may be controlled by multiple factors. The partial return to normal germination of chlamydospores produced by solopathogenic lines makes it difficult to account for the lethal on this basis. Chromosomal abnormalities preventing the normal disjunction of the chromosomes at meiosis, yet capable of a partial return to normal in the process of the reductional divisions, might possibly be the cause. This would account for the differences found among solopathogenic lines with respect to abnormal germination, their partial recovery, and the perpetuation of this recovery in the chlamydospores produced by crosses between compatible segregates from solopathogenic lines.

The correlation that occurs between abnormal germination and the increased size of the chlamydospores is unexplainable with the data available. It is interesting that Laskaris (13) found the same correlation in *Sphacelotheca sorghi.*

SUMMARY

Chlamydospores from certain crosses between monosporidial lines of *Ustilago zeae* germinate abnormally, producing gnarled and distorted promycelia that either autolyze before producing sporidia or produce relatively few sporidia in an irregular manner.

Extensive experiments indicate that the lysis is not caused by an infectious agent of the nature of bacteriophage.

The factor or factors for lysis are carried in certain lines only and segregation for factors affecting this character has been demonstrated by an appropriate series of crosses.

As the lysis is restricted to the promycelium, the operation of factors for lysis and other abnormalities associated with it seems to be restricted to the period during which meiosis occurs.

There was a definite tendency for the production of unusually large numbers of solopathogenic (apparently diploid) sporidia by promycelia of chlamydospores resulting from crosses involving one or more haploid lines carrying the factors for lysis.

Solopathogenic lines grew normally, caused infection when inoculated singly into corn plants, and in other respects behaved like similar lines described by other investigators.

Mutation occurs in solopathogenic lines, as well as in haploid lines.

There was partial reversion to the normal type of germination in the chlamydospores produced by some solopathogenic lines, as indicated by a decided decrease in the percentage of abnormal and disintegrating promycelia. When haploid sporidia, produced by such "reverting" lines were crossed, it was evident that the recovery persisted.

There is a correlation between the abnormally large size of chlamydospores and the tendency for these to germinate abnormally. This is unexplained, but, according to Laskaris, occurs in *Sphacelotheca sorghi,* also.

LOUISIANA STATE UNIVERSITY,
UNIVERSITY STATION, BATON ROUGE, LA.

LITERATURE CITED

1. CHILTON, S. J. P. The occurrence of lysis in certain crosses of *Ustilago zeae.* (Abstract) Phytopath. **28**: 5. 1938.
2. CHRISTENSEN, J. J. Mutation and hybridization in *Ustilago zeae.* Part II. Hybridization. Minn. Agr. Exp. Stat. Tech. Bull. 65. 1929.
3. ———. Studies on the genetics of *Ustilago zeae.* Phytopath. Zeitschr. **4**: 129–188. 1931.
4. DICKINSON, S. A method of isolating and handling individual spores and bacteria. Proc. Roy. Soc. Medicine **19**: 1–4. 1926.
5. EDDINS, A. H. Pathogenicity and cultural behavior of *Ustilago zeae* (Beckm.) Ung. from different localities. Phytopath. **19**: 885–916. 1929.

6. FISHER, R. A. Statistical Methods for Research Workers. 4th Ed. 307 pp. Oliver and Boyd, London and Edinburgh, 1932.
7. GOLDSCHMIDT, V. Vererbungsversuche mit den biologischen Arten des Antherenbrandes (*Ustilago violaceae* Pers.) Ein Beitrag zur Frage der parasitären Spezialisierung. Zeitschr. Bot. 21: 1–90. 1928.
8. HANNA, W. F. A simple apparatus for isolating single spores. Phytopath. 18: 1017–1021. 1928.
9. ————. Studies in the physiology and cytology of *Ustilago zeae* and *Sorosporium reilianum*. Phytopath. 19: 415–442. 1929.
10. ———— and W. POPP. Relationship of the oat smuts. Nature 126: 843–844. 1930.
11. HOLTON, C. S. Hybridization and segregation in the oat smuts. Phytopath. 21: 835–842. 1931.
12. KIENHOLZ, J. R., and F. D. HEALD. Cultures and strains of the stinking smut of wheat. Phytopath. 20: 495–512. 1930.
13. LASKARIS, T. A heritable lysis in germinating chlamydospores of *Sphacelotheca sorghi*. Phytopath. 31: 254–263. 1941.
14. RODENHISER, H. A. Studies on the possible origin of physiologic forms of *Sphacelotheca sorghi* and *S. cruenta*. Jour. Agr. Res. [U.S.] 49: 1069–1086. 1934.
15. SNEDECOR, G. W. Calculation and interpretation of analysis of variance and covariance. 96 pp. Collegiate Press, Inc. (Ames, Iowa). 1934.
16. STAKMAN, E. C., and J. J. CHRISTENSEN. Heterothallism in *Ustilago zeae*. Phytopath. 17: 827–834. 1927.
17. ————, ————, C. J. EIDE, and BJORN PETURSON. Mutation and hybridization in *Ustilago zeae*. Part I. Mutation. Minn. Agr. Exp. Stat. Tech. Bull. 65. 1929.
18. ————, L. J. TYLER, G. E. HAFSTAD, and E. C. SHARVELLE. Experiments in physiologic specialization and nature of variation in *Ustilago zeae*. (Abstract) Phytopath. 25: 34. 1935.

# EVIDENCE FOR THE EVOLUTION OF PHYTOPATHOGENIC VIRUSES FRO MMITOCHONDRIA AND THEIR DERIVATIVES.[1] II. CHEMICAL EVIDENCE

H. G. DuBuy and M. W. Woods

(Accepted for publication January 16, 1943)

## INTRODUCTION

Following the presentation of cytological and genetic evidence for the evolution of viruses from plastids or mitochondria, this paper presents chemical evidence for such evolution by demonstrating the occurrence of a ribose nucleoprotein in the plant plastid, of the same general type that constitutes viruses. There exist already indications of the presence of nucleoproteins outside the nucleus, especially in the plastids.

1. Plastids either derive from chondriocontes, specialized mitochondria or proplastids, which divide, or by division of plastids already present (10).

2. Variegation-inducing plastids, producing symptoms like viruses, show heredity of their abnormal characteristics. Banga (1) and Bawden (2) indicated the presence of an extranuclear hereditary substance in the plastid: a nucleoprotein.

3. Claude (3) demonstrated the presence of nucleoprotein in particles of protoplasmic centrifugates of animal origin, presumably chondriosomes or mitochondria.

4. Reed and Dufrenoy (13), Banga and Szent-Gyorgyi (1) demonstrated the presence of phosphorus in the plastid (although lecithins as phosphorus carriers might have been involved here).

There exist also some indications of a relation between these nucleoproteins and virus nucleoproteins.

1. Bawden *et al.* (2) were the first to prove the presence of ribose nucleic acid in virus-proteins. Previous experiments have demonstrated the interrelation between tobacco-mosaic virus and the chromoprotein complex (20).

2. They also have demonstrated the dependence of both chromoprotein and virus-protein on the same building stones and the same respiration system (21).

## EXPERIMENTAL

### I. Isolation of Chloroplasts in Suspension

In order to separate the chromoprotein complex from the nuclei and cytoplasmic proteins, the preparation of pure plastids was necessary. For the first steps the method of Granick (9) was used with modifications.

*Method.* 100 g. of leaves of *Nicotiana tabacum* var. Maryland commercial were ground for 2 minutes in a Waring blender in an 11 per cent sucrose – .5 per cent thiourea solution. (The behavior of plastids in solu-

[1] Scientific paper No. A43, Contribution No. 1870 of the Maryland Agricultural Experiment Station (Department of Botany).

tions from 2–22 per cent was studied. A 5 per cent sucrose solution, generally considered isotonic with the cell, preserves the plastids poorly. Admixtures of salts tend to break up the plastids.) The suspension was kept at about 10° C. throughout the experiment. It was filtered through cheese cloth and then through pyrex glass wool. (Cytoplasmic strands occur when the preparation is not strained through glass wool.) The suspension was then subjected to selective centrifugation. Each time the pellet and the supernatant were examined microscopically.

Three minutes of centrifugation with an Adams Senior Angle Centrifuge

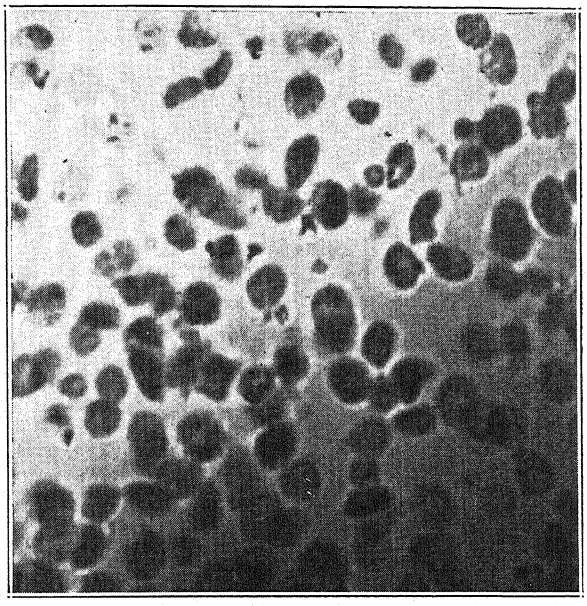


Fig. 1. Photomicrograph of purified plastid preparation of leaves of *Nicotiana tabacum* var. Turkish.

at about 1500 r.p.m. followed by 20 seconds at 3600 r.p.m. for packing threw down heavy débris, starch, plastids with starch, a great amount of triangular crystals, and nuclei. (Granick reports that nuclei disintegrate. Fortunately, in our material the nuclei were thrown down intact before the plastids.) The supernatant was centrifuged at about 4000 r.p.m. The pellet contained whole and some broken chloroplasts, and approximately 1 nucleus to each 1000 chloroplasts. The resuspended pellet was recentrifuged at low speed (1500 r.p.m.) and the new pellet discarded. The supernatant was recentrifuged at 4000 r.p.m. The pellet now contained mainly intact plastids (Fig. 1) with some plastid débris. The pellet was rewashed and recentrifuged. Preparations of this type are free of nuclei and débris and, if kept in the refrigerator at about 4° C., remain intact for months.

## II. Extraction of the Nucleoprotein

Preliminary extractions of nucleoprotein were made from preparations of the chromoprotein complex of the whole cell content, since the loss of material through purification of plastids amounts to 60–80 per cent.

A. *Extraction from Extracts of the Whole Cell.* The general method used for the preparation of the chromoprotein complex has already been described (20). However, some important modifications were made.

(1) The material was ground in a Waring blender in 0.1 per cent $Na_2HPO_4$ buffer and 0.5 per cent thiourea. *Thiourea has proved an excellent anti-oxidant.* Denny (5) was the first to use it to prevent the oxidation of phenolic compounds.

(2) The ammonium sulphate, distilled water, and other solvents used were all brought to pH 7–8 in order to avoid any great deviation from the pH range of cytoplasm. In certain cases dilute sodium dodecylsulphate (0.1 to 0.05 per cent) was used where it was desirable to break up particles. Its use will be specifically mentioned.

For the determination of nucleoprotein, the chromoprotein preparation made according to the previously described method (20) was precipitated by adding an equal volume of 30 per cent $(NH_4)_2SO_4$, collected on a Johns-Manville hyflo supercel celite filter, was washed twice with 15, 7.5, 5 and 2.5 per cent $(NH_4)_2SO_4$. The filtercake was then washed with distilled water, followed by elution with .1 per cent $Na_2HPO_4$. This procedure was repeated, and the chromoprotein finally eluted with distilled water, brought to pH 8. Twice the volume of acetone was added in order to remove the pigments. The precipitated protein mixture was reextracted repeatedly with acetone. With the third or fourth wash the carotenoids came off, since these were more tightly bound than the chlorophylls. The preparation was then resuspended in chloroform-ether to remove lipoids, washed with alcohol, and finally with distilled water.

Digestion of the whole chromoprotein complex was accomplished either by 0.5 N NaOH for 3 hours at room temperature or 18 hours at 0° C., or by 0.1 N KOH for 24 hours at 0° C. The preparation was then brought to pH 8 and centrifuged. The pellet was re-extracted with either NaOH or KOH and the two supernatants added.

The liberated nucleoprotein was precipitated by bringing the solution to pH 4.8 with dilute HCl, followed by centrifugation. The nucleoprotein pellet was repeatedly washed with water of pH 4.8.

The nucleic acid was obtained from this nucleoprotein preparation by several methods.

(1) By dissolving the nucleoprotein in a NaOH solution at pH 8 followed by the addition of 5 times the volume of glacial acetic acid and centrifugation of the nucleic acid. The pellet was washed successively in 1 per cent HCl, alcohol, ether, then dried and tested.

(2) By dissolving the nucleoprotein in NaOH at pH 8, adjusting the pH to 3.5 with HCl, and then adding 95 per cent ethanol and some ethyl ether

until a precipitate was formed. This precipitate was then treated as described for the precipitate in procedure (1).

(3) By refluxing the chromoprotein, obtained after acetone treatment with N/80 $NH_4OH$ at 100° C. for several hours. Under these conditions first the nucleoprotein was split off, followed by liberation of the nucleic acid. The latter was further hydrolyzed on continuous boiling to non-precipitable fractions.

B. *Extraction of Nucleoprotein from Purified Plastids.* Since the extracts of whole cells, obtained by the method as described above, yielded relatively large quantities of nucleoprotein (which was to be expected according to our working hypothesis), extractions were now made from purified plastids in order to eliminate any possible contamination with cytoplasmic or nuclear proteins. The methods as described under A were used beginning with the acetone extraction. After removal of the pigments, the minute disks or grana, imbedded in the stroma of the purified plastid centrifugate (see p. 767) are clearly visible. A few observations might be of interest:

(1) Alcohol extraction of the pigments was not possible. Apparently, the sucrose made the plastid wall impermeable and tough. Acetone allows extraction of the plastid pigments.

(2) In order to be sure that no pentose or phosphorus might be obtained from lecithin-like components of the chromoprotein complex, the acetone-washed preparation was not washed with chloroform, but refluxed for at least 1 hour in a chloroform-ether mixture, thereupon washed with ether, alcohol, and finally with distilled water.

(3) The nucleoprotein was extremely difficult to extract from the whole chromoprotein complex, when this complex had been beforehand precipitated either by freezing, boiling, or the addition of certain mineral salts. This indicates that any treatment that denatures the complex (for instance freezing of the material) might interfere with the tests for nucleoprotein or nucleic acid.

## III. Properties of the Chromoprotein Complex

A. *Light Absorbtion in the Visible Spectrum.* In order to prove that no appreciable denaturation occurred during the extraction of the chromoprotein complex or the preparation of purified plastids, extinction curves for visible light (3900–8000 Å) were made by means of a Coleman spectrophotometer. The spectral transmittance curve of a living leaf of *Nicotiana tabacum* var. Turkish (Fig. 2 "leaf") was compared with a chromoprotein extract of the same variety (Fig. 2 "chromoprotein"), and a purified plastid suspension in water (Fig. 2 "plastid"). The living leaf was infiltrated with water under evacuation immediately before measuring and placed in water between two microscope slides (14). The chromoprotein extract was one of those kept in a refrigerator for some months, to demonstrate the stability of the extracts. The region of maximum extinction for the chloro-

phylls in all three cases was 6780–6790 Å, for the carotenoids 4400 Å. From this evidence we conclude that no considerable denaturation of the natural chromoprotein extract occurred. That *both chlorophylls and carotenoids are carried by the same chromoprotein complex* is shown by the fact that the pigments remain together throughout the whole preparative procedure, and can be separated only by denaturation of the carrier complex (*cf.* 8). For comparison a curve showing the extinction by a petroleum-ether extract of the chromoprotein has been added (Fig. 2, "petroleum ether extr.").

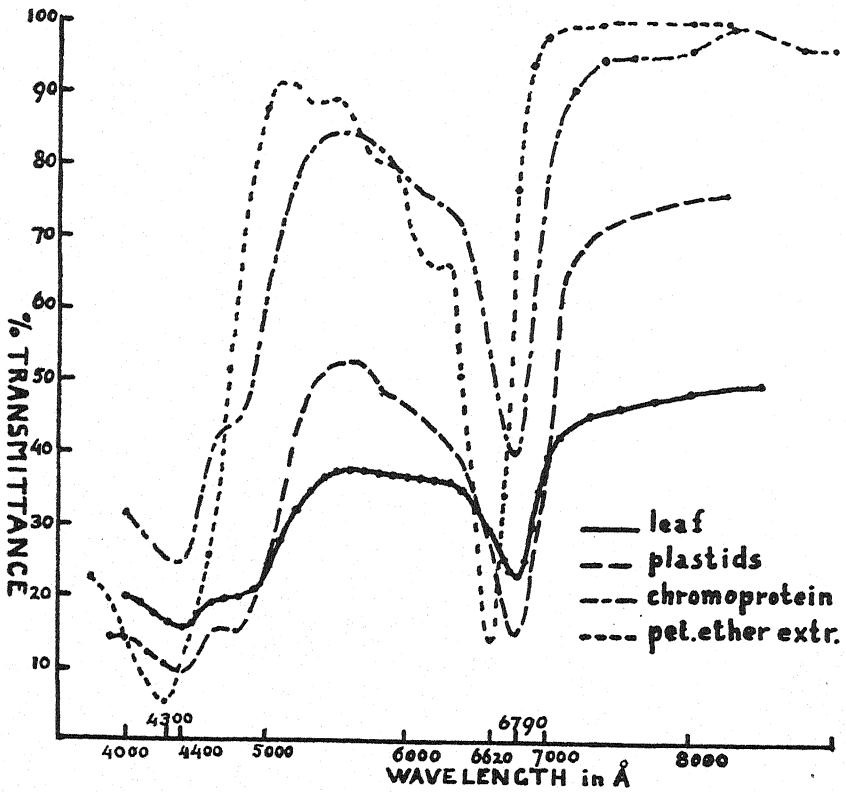



FIG. 2. Spectral transmittance curves in the visible spectrum of a living leaf of *Nicotiana tabacum* var. Turkish (unbroken curve). A purified plastid preparation of leaves of the same variety (widely broken curve), a chromoprotein solution of the same variety (dot-and-dash curve) and of a petroleum-ether solution of the same material (finely broken curve). Wavelength in Å-units, transmittance in percentage of water in the case of the living leaf and of the solvent in the others.

Since the prismatic dispersion and the light intensity of the lamp of the spectrophotometer change very gradually around 6780 Å, a very precise determination of the point of maximum extinction could be made by balancing the instrument for example at 6780 Å, and then adjusting the wavelength scale to the point where the deviation of the indicating galvanometer was minimal. In this manner the same value of maximum absorption for leaf and solutions has been determined independently by 3 workers in our

laboratory. The occurrence of carotenoids in chromoprotein extracts of spinach has already been mentioned by Smith (16) and others.

B. *Birefringence of the Chromoprotein Preparations as Compared with That of Mosaic-virus Preparations.* Chloroplasts have been reported as birefringent (10), whereas tobacco mosaic and certain other virus proteins show anisotropy of flow (2). Therefore, the purified chromoprotein extracts were tested for birefringence. Some of the purified preparations showed faint anisotropy of flow. Since the weakness of this phenomenon might be due to the occurrence of aggregates of mainly non-oriented micelles, a weak solution of sodium dodecylsulphate (0.1 per cent) or of $(NH_4)_2SO_4$ (1 per cent) was added in the proportion of 0.05 cc. per 10 cc. extract and the preparation allowed to stand for 24 hours at room temperature (about 22° C.). Strong green streaming birefringence was observed.

In order to determine whether this birefringence was due to the increased salt concentration itself or to its orienting effect the preparation was dialyzed against distilled water for 48 hours. Birefringence was still as strong as in the original sample, indicating that the salts acted only as orienting agents.

The absence of any contaminating masked virus in the extracts that, if present, might have caused birefringence was assured (1) by the method of fractionation that would have removed the more soluble virus proteins, (2) by infectivity tests on *Nicotiana tabacum* and *N. glutinosa,* which were negative, and (3) by differential precipitation of mosaic-virus protein and chromoprotein with a series of $(NH_4)_2SO_4$ concentrations.

The failure of the freshly prepared chromoprotein extracts to show streaming birefringence is apparently due to the fact that immediately after extraction the protein micelles are not oriented to form elongated compound particles. A similar situation was observed in tobacco-mosaic-virus preparations prepared from recently matured leaves shortly after infection. A lack of streaming birefringence of such virus preparations could be demonstrated by extracting the virus from young plants, infected only 12 days before extraction, following the method described previously (20), with the improvements as mentioned on page 768. These nonstreaming birefringent preparations, containing about 3.2 mg. protein per cc. of solution were colorless, only weakly opalescent, but highly infectious ("old" virus preparations are always somewhat brown). Various tests were performed with such preparations. One sample was left standing at room temperature. This developed weak birefringence in time. To a second sample sodium dodecylsulphate (to approximately 0.05 per cent) was added. Within 12 hours this developed strong streaming birefringence and also increased opalescence, which were identical in appearance to that occurring in samples of "old" virus. This sample, when kept for weeks, continued to show birefringence. A third sample was dialyzed against tapwater for 48 hours and distilled water for 48 hours. No streaming birefringence could be detected, even after 14 days standing at 4° C. A fourth nondialyzed sample

was kept at 4° C. This developed very weak streaming birefringence after some weeks standing. These observations are in accordance with Frampton's considerations on the shape and size of mosaic-virus particles (7).

Apparently, as in the case of the chromoprotein preparations, the micelles of "fresh" virus of recently infected plants *are not arranged in rod-shaped aggregates sufficiently to show streaming birefringence.*

Summarizing, we can state that removal of salt by dialysis suppresses the polar arrangement of the micelles in non-oriented preparations, whereas standing at room temperature increases the orientation slowly. Weak sodium dodecylsulphate solutions speed up the polar arrangement of the micelles.

C. *Fluorescence.* Watery solutions of the chromoprotein complex are clearly red fluorescent, although not so strongly as acetone solutions of pure chlorophyll.

In the light of the chemical and physical data presented here we can conclude that the chromoprotein complex, prepared by the methods given in this and a previous paper (20) must resemble closely the state in which it occurs in the living leaf.

## IV. Properties of the Nucleoprotein Obtained from Plastids

The nucleoprotein, obtained as described under III was soluble in alkaline solutions and precipitated at pH 4.7. It was insoluble in alcohol, acetone, ether and chloroform. The protein in solution had a high buffer capacity to base on the acid side of pH 4.7. Millon's, xanthoproteic, and biuret reactions were positive. Bial's test (orcin, HCl, $FeCl_3$) yielded a blue-green color reaction, the orcin-HCl test a purple color. The ammonium molybdate-benzidine reaction for phosphorus was positive. The nucleoprotein remained in solution even after 4 days of dialysis against distilled water. The solution was brownish and opalescent. The ultra-violet absorption spectrum was determined from a sample prepared from pure plastids by digestion of the protein complex (after removal of the pigments) with 0.1 N KOH at 0° C. for about 12 hours. The digest was brought to pH 6, the non-soluble protein centrifuged out and the supernatant brought to pH 8, diluted ten times and measured. The maximum absorption band was from 2570–2600 Å (Fig. 3).[2] The curve is similar to the curve obtained by Claude (3) for the tumor fraction of mouse-brain preparations, and by Bawden for bushy-stunt virus (2).

The pure nucleic acid preparation, obtained by alkaline digestion of the nucleoprotein was precipitated by 85 per cent acetic acid. The white powder was soluble in alkali at pH 8.0. Millon's, xanthoproteic, and biuret reactions were negative, as was Schiff's fuchsin-sulphurous acid test for desoxypentose. Schiff's aniline acetate test for furfural was positive after acid hydrolysis of the nucleic acid brought about by boiling it for a few

[2] We are indebted to Mr. H. Wiseman of the Bureau of Animal Industry, U.S.D.A., for determining the absorption spectrum of this preparation.

minutes in 12 per cent HCl, indicating the presence of d-ribose. The nucleic acid is totally hydrolyzed by 0.5 N NaOH in the cold or by boiling with dilute NaOH or with 30 per cent HCl. It is insoluble in boiling ether-chloroform or in ether-chloroform-alcohol mixture, even after continued refluxing. This excluded the presence of lecithin-like substances. A nucleic

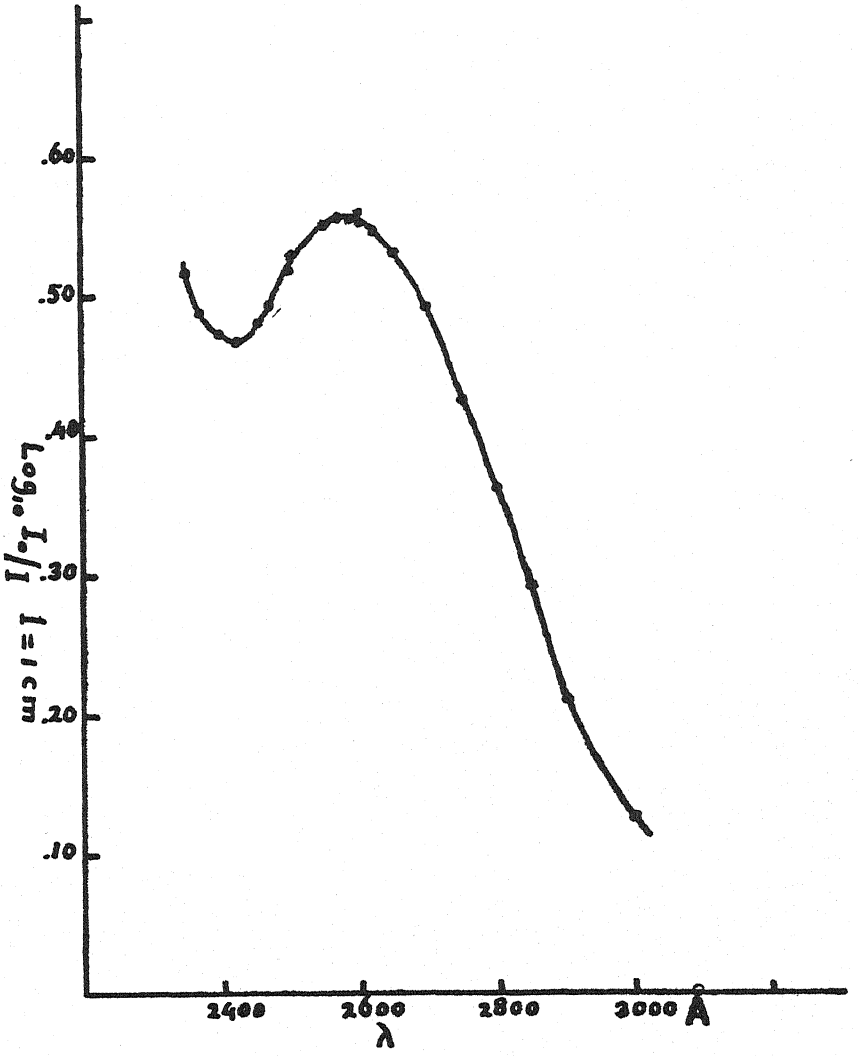



Fig. 3. The ultraviolet absorption spectrum of a nucleoprotein solution obtained from purified plastids of *Nicotiana tabacum* var. Turkish.

acid precipitate was obtained by hydrolyzing the chromoprotein complex with 0.5 N NaOH and by treating the alkaline solution of nucleoprotein (twice refluxed in ether with 5 volumes of glacial acetic acid). The precipitate was washed with 1 per cent HCl, alcohol, and ether, and then dried. After redissolving in dilute NaOH, the preparation was filtered and mea-

sured. It might have contained some nucleoprotein. The absorption curve was determined according to the usual match-point method.[3] Maximum absorption was between 2560 and 2600 Å.

## DISCUSSION

A number of investigators have worked with "Lubimenko-extracts" (12), which are partly suspensions of the grana that constitute the chloroplasts, and with various chlorophyll extracts. Hubert (11) reviews the literature before 1935. He reports that, except for a ground leaf suspension, the main absorption band of chlorophyll in colloidal solutions is always found moved towards the blue. Since 1935, Stoll and Wiedemann (17), Shafer (15), Smith (16), Fishman and Moyer (6), to mention a few, have worked with modified Lubimenko extracts of chlorophyll. They mention a brown supernatant that indicates oxidation, which we have shown to cause an instability of the position of the absorption bands of the purified pigmented protein extracts. Our experiments have demonstrated that this instability is due to an insufficient prevention of the action of oxidative enzymes. Some of the samples prepared according to the methods described in this paper have been stable for more than 6 months when kept in the refrigerator at about 4° C., showing only some broadening of the absorption bands. However, as soon as some oxidation was allowed to proceed, the point of maximum absorption shifted gradually from 6780 Å and after some hours to 6750 Å.

In an earlier paper the writers (22) presented cytological and genetic evidence for the evolution of viruses from plastids or proplastids (mitochondria or chondriocontes). In this paper the chemical similarity of viruses and an essential part of the chromoprotein complex, namely a ribose nucleoprotein is demonstrated. Thus, one of the arguments in favor of the organismal origin of viruses, namely, that no nucleoprotein of the ribose type could be found in the "host" in sufficient quantity to explain the high concentration of virus protein, sometimes encountered, is invalidated.

A further parallelism between the cytological and chemical evidence for the evolution of viruses from plastid or mitochondrial ribose-nucleoproteins can be found in the gradual modification of the variegation-inducing plastid as presented in a "spectrum" of variegations (22). The first cytological evidence of plastid change occurs in mild variegations. Here light green plastids are found. Continuing along the spectrum of variegation the next change is the occurrence of light-green plastids, which turn yellow (= loss of chlorophyll), the occurrence of yellow plastids, which bleach (= loss of some carotenoid pigment), the occurrence of colorless plastids (= loss of both chlorophylls and carotenoids), the occurrence of proplastids (chondriocontes or mitochondria) only (= loss of complex plastid structure), and finally, the occurrence of small sub-mitochondrial units only. Presumably slow-moving

[3] We are indebted to Mr. H. Armstrong of the National Cancer Institute for photographing the ultra-violet absorption spectrum of this sample.

viruses, such as that causing *Abutilon* mosaic, are of such a structure, whereas viruses like that of tobacco mosaic represent only the modified monomolecular ribose nucleoprotein residues of the plastid complex.

This gradual loss of components presents a parallel with the degrees of chemical linkage that exist between the components of the chromoprotein complex as indicated, *e.g.*, by the gradual breakdown of purified plastid preparations by controlled chemical means or by aging. A treatment with acetone removes the chlorophylls first, followed after repeated washing by the removal of the carotenoids. In order to remove the lecithin-like components still more drastic treatments must be used, and, in order to liberate the free nucleoprotein, a prolonged digestion is necessary.

Following up this parallel we offer the diagram below, part of which has already been proved to be correct, as a working hypothesis that visualizes the chemical evolution of viruses from normal plastids or mitrochondria.

TABLE 1.—*Diagram indicating the chemical evolution of plant viruses from plastids or mitochondria*

| Normal plastids | Plastids of Region I of the "variegation spectrum" | Mitochondria or proplastids of Region III–IV–V of the spectrum and certain complex viruses | Viruses |
|---|---|---|---|
| Chlorophylls | Carotenoids | Lipoproteins | Nucleoproteins of the ribose type |
| Carotenoids | Lipoproteins | Nucleoproteins of the ribose type | ..... |
| Lipoproteins | Nucleoproteins of the ribose type | ..... | ..... |
| Nucleoproteins of the ribose type | ..... | ..... | ..... |

The animal viruses of the ribose nucleoprotein-type could similarly have derived from the chondriome, or mitochondrial-complex of the host-cell. Thus, also over the chondriome a connection might be established between certain cancer agents and viruses (*cf.* 18). In this way the variegation-inducing plastid or chondrioconte can be considered analogous to the cancer inducing mitochondrium (= chondriosome) with its limited intercellular mobility. The work of Claude (3) presents evidence for this statement, since he has demonstrated the presence of ribose nucleoproteins in the particulate components of normal and cancerous cells of chick and mouse (presumably mitochondria) and has suggested the etiologic significance of abnormal mitochondria in cancer (*cf.* also 19).

Proof for the origin of animal viruses from the mitochondria or chondriome is more difficult to furnish, than for the origin of plant viruses, since the mitochondria in animals remain small and are never "tagged" like the modified plastids of plant-variegations. A further study of the causes leading to the gradual change of the nucleoprotein complex in the modified plant

plastids might lead to a better understanding of various animal viroses and cancers, as well.

SUMMARY

An extraction method for the chromoprotein complex from plastids is presented.

Some conditions for the occurrence of streaming birefringence of the complex are given.

For comparison some conditions for the occurrence or absence of streaming birefringence of tobacco mosaic virus protein are presented.

The resemblance of the chromoprotein complex to the complex as it occurs in the living state is demonstrated by analysis of the spectral absorption of extracts and living leaves and by some of its chemical and physical characteristics.

Various reactions characteristic of ribose nucleoprotein are given by a protein fraction of the chromoprotein complex of purified plastids of *Nicotiana tabacum*.

Some evidence for the evolution of plant virus nucleoproteins from the nucleoproteins of the plastids is presented, which suggests the possibility of a similar evolution of certain animal viruses from mitochondria, and a parallelism of variegation-inducing plastids and cancer-inducing mitochondria.

The authors wish to express their indebtedness to Dr. N. L. Drake, Head of the Department of Chemistry, University of Maryland, for his evaluation of the various chemical procedures used, and Miss H. Ryan for her help in preparing the manuscripts of the two papers.

LITERATURE CITED

1. BANGA, G., and A. SZENT-GYORGYI. Structure-proteins. Science 92: 514–515. 1940.
2. BAWDEN, F. C. Plant viruses and virus diseases. 272 pp. Chronica Botanica Co. (Leiden). 1939.
3. CLAUDE, A. Particulate components of normal and tumor cells. Science 91: 77–78. 1940.
4. COMAR, C. L. Chloroplast substance of spinach leaves. Bot. Gaz. 104: 122–127. 1942.
5. DENNY, F. E. Thiourea prevents browning of plant tissues and juices. Contr. Boyce Thompson Inst. 7: 55–61. 1935.
6. FISHMAN, M., and L. S. MOYER. Electrophoresis of the chlorophyll-protein complex. Science 95: 128–129. 1942.
7. FRAMPTON, V. L. On the size and shape of the tobacco mosaic virus protein particle. Science 95: 232–233. 1942.
8. FRENCH, C. S. The pigment-protein compound in photosynthetic bacteria. Jour. Gen. Physiol. 23: 469–482 and 483–494. 1940.
9. GRANICK, S. Quantitative isolation of chloroplasts from higher plants. Amer. Jour. Bot. 25: 558–560. 1938.
10. GUILLIERMOND, A. The cytoplasm of the plant cell. 247 pp. Chronica Botanica Co. (Waltham, Mass.). 1941.
11. HUBERT, B. The physical state of chlorophyll in the living plastid. Rec. trav. bot. néerl. 32: 323–390. 1935.
12. LUBIMENKO, V. Les pigments des plastes et leur transformation dans les tissus vivants de la plante. Rev. Gén. Bot. 39: 619–637. 1927.
13. REED, H. S., and J. DUFRENOY. Catechol aggregates in the vacuoles of cells of zinc-deficient plants. Amer. Jour. Bot. 29: 544–551. 1942.

14. Reinke, J. Die optischen Eigenschaften der gruenen Gewebe und ihre Bedeutung zur Assimilation des Kohlenstoffes. Ber. d. D. Bot. Ges. 1: 395. 1883.
15. Shafer, J. Solutions of chlorophyll in salt water. Science 91: 580. 1940.
16. Smith, E. L. The chlorophyll-protein compound of the green leaf. Jour. Gen. Physiol. 24: 565–598. 1941.
17. Stoll, A., and E. Wiedemann. Chlorophyll. Fortschr. Chem. Org. Naturstoffe 1: 159–238. 1939.
18. Strong, L. C. Crossroads in cancer research. Jour. Hered. 31: 471–474. 1940.
19. Taylor, A. R., D. G. Sharp, D. Y. Beard, and J. W. Beard. Properties of the isolated macromolecular component of normal chick embryo tissue. Science 94: 613–615. 1941.
20. Woods, M. W., and H. G. DuBuy. Synthesis of tobacco-mosaic virus protein in relation to leaf chromoprotein and cell metabolism. Phytopath. 31: 978–990. 1941.
21. ———. The effect of tobacco-mosaic virus on cellular respiration. Phytopath. 32: 288–302. 1942.
22. ———. Evidence for the evolution of phytopathogenic viruses from mitochondria and their derivatives. I. Cytological and genetic evidence. Phytopath. 33: 637–655. 1943.

# PRODUCTIVITY OF MOSAIC-RESISTANT REFUGEE BEANS

J. C. WALKER AND J. P. JOLIVETTE

(Accepted for publication December 18, 1942)

## INTRODUCTION

Since the variety Stringless Green Refugee has become a widely used snap bean (*Phaseolus vulgaris* L.) for canning in northern United States, its extreme susceptibility to common mosaic (*bean virus 1* Pierce) has often been a limiting factor in production. The successful control of the disease in this type of bean began with the discovery of an immune plant in the variety by Ralph Corbett of the Sioux City Seed Company at Billings, Montana, in 1929. The progeny of this plant was also completely immune, and the subsequent variety known as Corbett Refugee has served as a parent for breeding purposes. In itself Corbett Refugee, although similar to Stringless Green Refugee in many respects, was unsuitable in others to replace the latter. The first 2 varieties developed from Stringless Green Refugee and Corbett Refugee were derived from a cross made in 1930 by Pierce and Walker (8). These were designated as Wisconsin Refugee and Idaho Refugee and were introduced to the trade in 1934. A third variety was introduced as U. S. No. 5 Refugee by Wade and Zaumeyer (11) in 1935. In 1941 Anderson (1) announced two new varieties, Sensation Refugee 1066 and Sensation Refugee 1071, derived from a cross of Idaho Refugee with Stringless Green Refugee and with U. S. No. 1, respectively.

By 1937 the first three of the resistant varieties mentioned had reached commercial volume. Preliminary experimental lots of the last two varieties were made available by the introducers. The purpose of the investigation reported herein was to study the productivity, pod type, and canning quality of these new varieties in comparison with mosaic-susceptible Stringless Green Refugee. Some attention was also given to the effect on yield of an inherited variegation in some of the varieties.

## METHODS AND MATERIALS

Trials were conducted at Madison, Wisconsin, in 1937, 1938, 1939, and 1942, and at Racine and Green Bay, Wisconsin, in 1939. Single-row plots, 25 feet long, were planted in randomized and replicated plots at Madison and Racine. Double-row, non-replicated plots, 150 feet long, were planted at Green Bay. Pods were picked at regular intervals and weighed. Since no significant differences occurred between various commercial lots of a given variety, only the averages for each variety were presented here. Commercial lots of Stringless Green Refugee usually contain a majority of seeds that carry *bean virus 1*. Before mid-season most plants in this variety became diseased. In order to study the effects of the disease on this variety, a virus-free stock was built up by growing a commercial stock in the greenhouse where infected plants could be eliminated and dissemination

of the virus prevented. The stock thus secured was increased out of doors in an isolated area and again rogued thoroughly. The mosaic-free stock was used in the yield trials of 1939 and 1942.

At certain pickings in 1939 random samples from each lot were graded according to standard sizes based on the maximum diameter of the pod. Since the ideal Refugee bean pod for canning is circular in cross section, measurements also were made of the diameter of the cross section taken from cheek to cheek and from suture to suture. The S/C factor was then obtained by dividing the average cheek diameter into the average suture diameter. Thus, as pods tended to become flat this factor exceeded 1.00. The writers are indebted to Dr. J. H. Torrie of the Department of Agronomy, University of Wisconsin, for assistance in selection of plot designs and in setting up data for analysis.

## EXPERIMENTAL RESULTS

### Occurrence of Mosaic-like Variegation

Corbett Refugee contains a chlorophyll-deficient character that has been retained in Wisconsin Refugee and Idaho Refugee. It is manifested in various forms some of which are readily confused with mosaic. Mottling occurs in one or more leaflets of the trifoliate, sometimes in the unifoliate leaf, and very commonly it is confined to one side of the midrib, causing dwarfing, which may be so extreme as to result in pronounced unilateral distortion. Occasional plants may show extreme deformity of most leaves while pods are distorted, misshapen, and disfigured by necrotic areas. On the other hand, in a large share of the plants affected the abnormality is confined to 1 or 2 leaves which may drop early, leaving the plant normal for the remainder of the season.

Harrison and Burkholder (3) and Horsfall, Reinking and Burkholder (4) described this abnormality in New York and suggested it to be virous in nature, since it appeared first in the younger leaves. Zaumeyer (12) has shown it to be a hereditary characteristic, and this has been confirmed repeatedly by the writers. Other hereditary chlorophyll deficiencies in bean have been described (5, 6, 7, 9) which might be confused with mosaic. None of these seem to conform with the character found in strains of Refugee in which Corbett Refugee has been a parent. Zaumeyer (12) has presented evidence to show that it consists of 2 very similar types of variegation, and that one type of variegation is controlled by 2 complementary factors. However, no progenies breeding true for this character were secured indicating either that the double recessive is lethal or that modifying genes prevent or obscure the expression of the character in some plants of the double recessive progenies. Wade (10) has recently analyzed a second type in U. S. No. 5 Refugee very similar to that found in Corbett Refugee, Wisconsin Refugee, and Idaho Refugee. He suggests that this may have arisen in U. S. No. 5 Refugee as a result of a chance cross with Corbett Refugee. He interprets that this type of variegation may be due to any of

3 recessive genes, while the normal is attributable to the complementary action of 3 dominant genes.

Certain studies were made of single-plant progenies from plants of Wisconsin Refugee and Idaho Refugee with no abnormality and from plants showing various grades of variegation. No attempt has been made to distinguish between the 2 types indicated by Zaumeyer (12) and Wade (10). Selections made in 1939 were tested at Madison in 1940. The results in table 1 show that an average of 20 per cent of all plants were affected in the commercial Wisconsin Refugee stock, and approximately the same percentage occurred in progenies from plants selected as free from variegation. However, progenies from moderately and severely affected parents showed corresponding increases in percentages of total plants affected and in relative proportions of moderately and severely affected individuals. Wade (10) points out that in field planting of Corbett Refugee at Charleston, South Carolina, only about 25 per cent showed variegation,—in such cases the degree was slight. Under greenhouse conditions, however, all Corbett Refugee plants were variegated, and the mean was very close to that of the three lines carrying double recessives. Further evidence that variegation in Wisconsin Refugee is influenced by environment is shown in a greenhouse experiment conducted during winter months at Madison, Wisconsin.

TABLE 1.—*The occurrence of inherited variegation in progenies from plants selected from Idaho Refugee and Wisconsin Refugee and in commercial Wisconsin Refugee*

| Degree of abnormality in parent plants | Number plants in progenies tested | Percentage of plants showing degree of abnormality indicated | | | |
|---|---|---|---|---|---|
| | | None | Slight | Moderate | Severe |
| None | 118 | 83 | 10 | 3 | 4 |
| Moderate | 173 | 29 | 18 | 22 | 31 |
| Severe | 165 | 15 | 7 | 20 | 58 |
| Wis. Refugee (Comm.) | 149 | 80 | 9 | 9 | 2 |

Plants from commercial Wisconsin Refugee and from seed selected from chlorophyll-deficient plants of that variety were grown in the greenhouse at 4 temperature levels: 16, 20, 24, and 28° C. The results, given in table 2, show that the lot grown from seed of affected parents contained a high percentage of affected individuals at 16, 20, and 24° C., while the abnormality was very decidedly suppressed in this group and in commercial Wisconsin Refugee at 28°. It may be possible, therefore, for variation in the amount of this chlorophyll aberration to be found in fields from the same lot of seed due to temperature variations resulting from differences in planting date, season, and location. Furthermore, it was found necessary to make frequent counts on this character to record all affected plants since only 1 or 2 leaves might show the abnormality, while earlier and later leaves remained normal. It is important, therefore, that these facts be taken into account in the genetic analysis of this character. By selection within Idaho Refugee, lines free of

this character have been obtained. No evidence of this abnormality was noted in lots of Stringless Green Refugee, U. S. No. 5 Refugee, Sensation Refugee 1066, and Sensation Refugee 1071 used in these experiments.

TABLE 2.—*The occurrence of variegation in commercial Wisconsin Refugee and in progenies from affected plants in this variety when grown in the greenhouse at different air temperatures at Madison, Wisconsin, in December, January, and February*

| Average air temperature of greenhouse | Progeny from affected plants of Wisconsin Refugee | | Commercial Wisconsin Refugee | |
|---|---|---|---|---|
| | Plants tested | Plants affected | Plants tested | Plants affected |
| *°C.* | *Number* | *Per cent* | *Number* | *Per cent* |
| 16 | 40 | 60.0 | 33 | 15.1 |
| 20 | 51 | 64.7 | 35 | 17.1 |
| 24 | 52 | 50.9 | 30 | 16.7 |
| 28 | 37 | 8.1 | 32 | 3.1 |

## Total Yields

The average yields for each strain or variety are given in table 3. In 1937 commercial Stringless Green Refugee gave the lowest yields, while Wisconsin Refugee was consistently intermediate between it and Idaho Refugee. U. S. No. 5 Refugee yielded closely to the latter in both seasons, while in 1938 Sensation Refugee 1066 and Sensation Refugee 1071 produced much more heavily than all others. In 1939 essentially the same order was maintained at 3 locations in the State with the exception that Wisconsin Refugee fell below Stringless Green Refugee at Madison. Idaho Refugee, U. S. No. 5 Refugee, Sensation Refugee 1066 and 1071 were each significantly higher in production than Wisconsin Refugee at this location, but there was no significant difference between the 4 highest yielders. At Racine these 4 were again highest, but only one (Sensation Refugee 1071) was significantly higher than Wisconsin Refugee. At Green Bay, where only a single plot per variety was planted, the Wisconsin Refugee yield was higher than that of either Idaho Refugee or U. S. No. 5 Refugee, while Sensation Refugee 1066 and 1071 were again the highest yielding lots.

It is of special interest to note the comparative yields of Stringless Green Refugee from commercial and from specially selected mosaic-free seed. These were compared in 1939 and in 1942. In the former year mosaic occurred in a large percentage of the plants from the commercial seed, while those plants from mosaic-free seed remained free from visible current-season infection until well into the harvest. In 1942, by contrast, there was early dissemination of the virus from the commercial stock to the selected stock with the result that both were severely affected before harvest. In 1939 the mosaic-free stock produced as heavily as Idaho Refugee, U. S. No. 5 Refugee, Sensation Refugee 1066, and Sensation Refugee 1071, and significantly higher than the commercial stock. In 1942, when the virus was disseminated early and the disease was equally severe in both lots, the selected lot dropped in yield close to the level of the commercial stock.

TABLE 3.—*Yield of mosaic-resistant and mosaic-susceptible varieties of bean of the Refugee type in 1937, 1938, 1939, and 1942*

| Variety | Yield in pounds per acre at | | | | | |
|---|---|---|---|---|---|---|
| | Madison in | | | | Racine in | Green Bay in |
| | 1937 | 1938 | 1939 | 1942 | 1939 | 1939 |
| Stringless Green Refugee (Commercial) | 2160 | 4300 | 7457 | 6570 | 6458 | 4770 |
| Stringless Green Refugee (mosaic-free seed) | ..... | ..... | 10085 | 6711 | 8256 | 5880 |
| Corbett Refugee | ..... | 7380 | ..... | ..... | ..... | ..... |
| Wisconsin Refugee (Commerial) | 3058 | 5400 | 7066 | ..... | 7727 | 6180 |
| Wisconsin Refugee (selected for variegation) | ..... | 5920 | 8290 | ..... | 7426 | ..... |
| Idaho Refugee (Commercial) | 4883 | 7120 | 9736 | 11224 | 8542 | 5675 |
| Idaho Refugee (free from variegation) | ..... | ..... | ..... | 11813 | ..... | ..... |
| U. S. No. 5 Refugee | 4931 | 6570 | 10174 | 10743 | 7939 | 5945 |
| Sensation Refugee 1066 | ..... | 9220 | 11282 | 9843 | 8265 | 7410 |
| Sensation Refugee 1071 | ..... | 9080 | 10052 | 12807 | 12808 | 6900 |
| Difference required for significance (19:1) | 855 | 1280 | 1520 | 3050 | 1220 | ..... |

In 1938 and 1939 a lot of Wisconsin Refugee was included that had been derived from seed selected from plants showing the variegation character described above. About 85 per cent of the plants in this stock showed the character. It is to be noted that no significant difference occurred between the yield of this lot and that of the commercial stock of the variety. In 1942 a stock of Idaho Refugee, free from this variegation as a result of several years' selection, was included. The yield of the variegation-free stock was not significantly different from that of the commercial stock of the variety. It may be concluded from these data that the variegation character has little or no influence upon yield. Its chief detrimental effect is the fact that occasional misshapen pods unsuitable for canning occur. The varieties possessing this character can and should be rid of it by selection.

## Rate of Production

The rate of production as shown by the yield at various picking dates is of great importance in evaluating a variety for canning purposes. In figures 1 and 2 are given the yield curves for each variety tested at Madison in 1937, 1938, 1939, and 1942. The season of 1937 was very dry in late July and throughout August. In 1938 and 1942 the moisture supply was ample and well distributed during the picking season. In 1939 the rainfall, though not heavy, was well distributed, and in this year it was supplemented at Madison by overhead irrigation. This resulted in a longer picking period, so that harvest continued until September 11, although only yield data through August 28 are given in figure 1.

The commercial stock of Stringless Green Refugee rose gradually to a peak after mid-season in each year and with Wisconsin Refugee was usually

latest in initial yields of substantial size. In Corbett Refugee, tested only in 1938, the first pick also coincided with Stringless Green Refugee and the yield rose gradually to a peak about mid-season. Idaho Refugee and U. S.

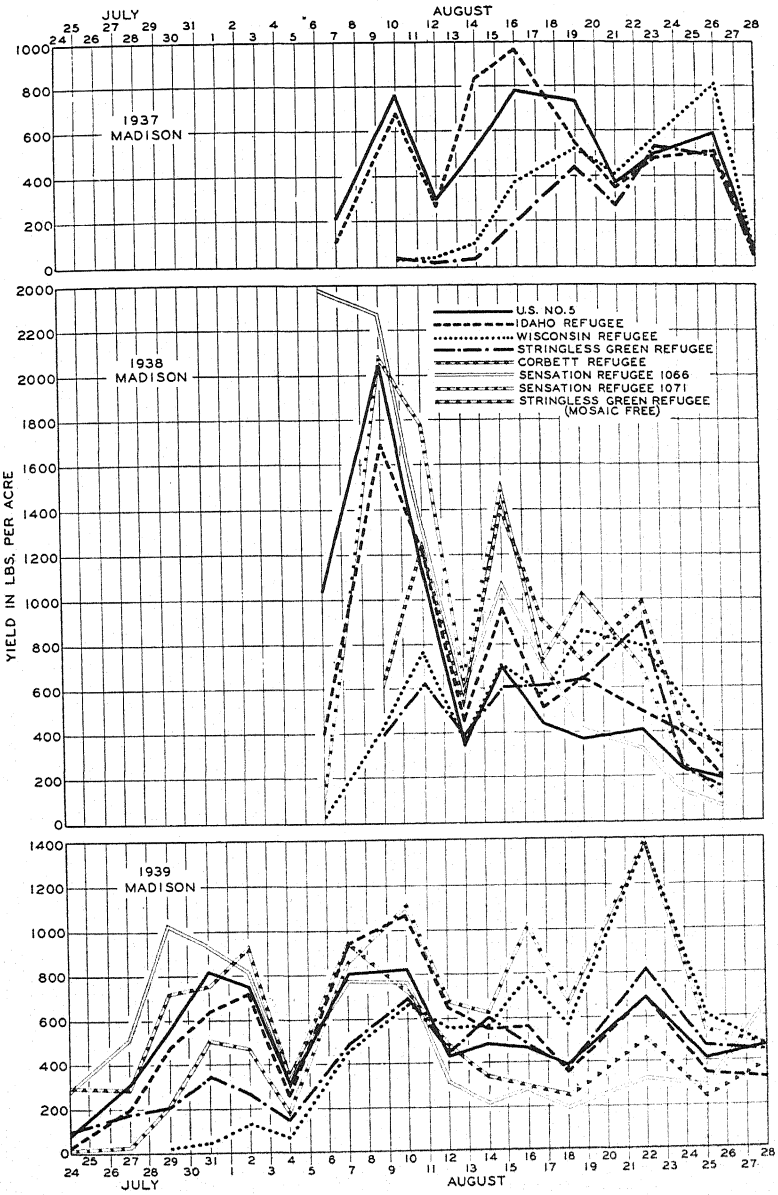



FIG. 1. Rate of production of resistant and susceptible varieties at Madison, Wisconsin, in 1937, 1938, and 1939.

No. 5 Refugee, on the other hand, rose to early harvest peaks. Thus, it might appear that the former of these was much earlier in initial pick and harvest peak than either parent, Stringless Green Refugee or Corbett

Refugee. However, mosaic-free Stringless Green Refugee when grown quite free from mosaic in 1939 produced a yield curve very similar to that of Idaho Refugee, and one that is undoubtedly the heritable yield characteristic of the variety.

Wisconsin Refugee, although it usually yielded on a curve nearly parallel with Stringless Green Refugee, being free from the influence of mosaic, is actually a later variety. Thus, of the two varieties derived from the cross between Stringless Green Refugee and Corbett Refugee, one, Wisconsin Refugee, resembles in maturity and yielding habit the resistant parent, while the other, Idaho Refugee, resembles the susceptible parent.

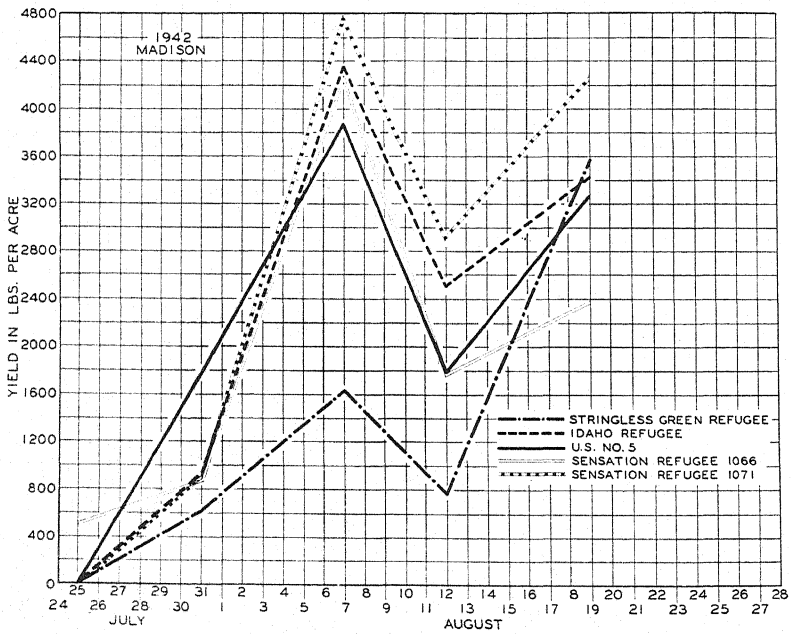



FIG. 2. Rate of production of resistant and susceptible varieties at Madison, Wisconsin, in 1942.

Sensation Refugee 1071 reached an early peak along with U. S. No. 5 Refugee, Idaho Refugee, and mosaic-free Stringless Green Refugee in 1938 and 1942, but the production curve rose more gradually in 1939. Sensation Refugee was usually ready for the first pick 4 to 7 days ahead of the other strains tested.

The mosaic disease causes some blossom-drop, stunts the plant, and delays maturity of pods. The effect of this disease on the rate of production of Stringless Green Refugee is shown when the picking curves for 1939 and 1942 are compared (Fig. 3). It has been pointed out that in the former year the mosaic-free stock remained free from disease until late in the season, while in 1942 plants from the identical lot became infected early through current-season spread. In 1939 the planting from mosaic-free seed reached

an early production peak while in 1942 when the plants were affected early it reached a late-season production peak along with that usual for the commercial stock. It appears that the high productivity of certain of the resistant varieties is due primarily to the control of mosaic through introduction of the resistant character by hybridization rather than by the introduction of any other factors that induced higher yields.

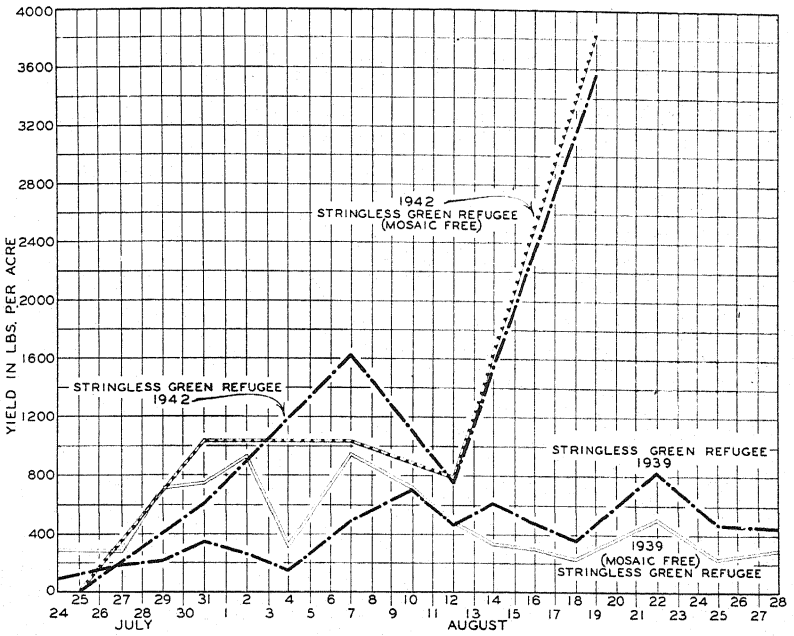



FIG. 3. Comparison of commercial Stringless Green Refugee production rate with that of the same variety grown from mosaic-free seed. In 1939 the latter remained free from symptoms for most of the season and reached a production peak much earlier than the former. In 1942, when mosaic spread early throughout the plantings, both stocks reached a production peak late in the season.

## Relative Pod Size

At several pickings at Madison in 1939 random samples from the harvested pods were taken from each variety and sorted into the standard-size classes used by canners. The results in table 4 show that the crop was picked so as to give a large proportion of small sizes, as desired for the whole bean pack, for which this type is particularly suited. It is to be noted that there was little variation between varieties in the 3 and 5 sizes. Most lots were quite similar to Stringless Green Refugee in 1–2 and 4 sizes. U. S. No. 5 Refugee was significantly lower than the former and Idaho Refugee in the percentage of pods in the 1–2 size.

## Relative Pod Shape

It is highly desirable that beans of this type be practically circular in cross section. In 1939 random samples of 25 pods were taken from 2 pick-

ings of each lot at Racine and Madison, and the S/C ratio was determined. The data secured are given in table 5. These results show that the pods were significantly flatter at each station in the first as compared with the second picking. However, when Stringless Green Refugee is compared with any other variety at any one of the pickings, the difference is not significant, except in one picking of U. S. No. 5 Refugee. It may be concluded, therefore, that all of the mosaic-resistant varieties tested are equal in this important character to the mosaic-susceptible parent variety.

TABLE 4.—*Relative proportion of standard pod sizes at 5 pickings at Madison in 1939*[a]

| Variety | Averages of percentage of pods by weight in the indicated size class | | | |
|---|---|---|---|---|
| | 1–2 | 3 | 4 | 5 |
| Stringless Green Refugee (Commercial) | 46 | 36 | 15 | 3 |
| Stringless Green Refugee (mosaic-free seed) | 39 | 36 | 20 | 5 |
| Idaho Refugee | 46 | 31 | 21 | 2 |
| U. S. No. 5 Refugee | 33 | 38 | 25 | 4 |
| Sensation Refugee 1066 | 38 | 37 | 22 | 3 |
| Sensation Refugee 1071 | 40 | 34 | 23 | 3 |
| Wisconsin Refugee[a] (Commercial) | 48 | 34 | 17 | 1 |
| Wisconsin Refugee (free from variegation) | 43 | 34 | 22 | 1 |
| Difference required for significance (19: 1) | 11 | [b] | [b] | [b] |

[a] Wisconsin Refugee was later in maturity than the other varieties; therefore, only 3 pickings of lots of this variety were included, and it was not included in the analysis of variance.

[b] Differences not significant.

TABLE 5.—*Pod shape as indicated by the S/C factor*

| Variety | S/C factor at station and dates indicated | | | | | |
|---|---|---|---|---|---|---|
| | Racine | | | Madison | | |
| | Aug. 17 | Aug. 20 | Means | Aug. 18 | Aug. 25 | Means |
| Stringless Green Refugee (Commercial) | 1.08[a] | 1.01 | 1.05 | 1.07 | 1.01 | 1.04 |
| Stringless Green Refugee (mosaic-free seed) | 1.07 | 1.00 | 1.04 | 1.06 | 1.10 | 1.08 |
| Idaho Refugee | 1.04 | 1.00 | 1.03 | 1.02 | 0.99 | 1.00 |
| U. S. No. 5 Refugee | 1.14 | 1.03 | 1.09 | 1.06 | 0.94 | 1.00 |
| Sensation Refugee 1066 | 1.07 | 1.02 | 1.05 | 1.05 | 1.05 | 1.05 |
| Sensation Refugee 1071 | 1.04 | 1.00 | 1.02 | 1.01 | 0.97 | 0.99 |
| Wisconsin Refugee (Commercial) | 1.05 | 1.03 | 1.04 | 1.03 | 0.99 | 1.01 |
| Wisconsin Refugee (selected for variegation) | 1.05 | 1.04 | 1.05 | 1.05 | 0.97 | 1.01 |
| Means | 1.07[b] | 1.02 | ..... | 1.04 | 1.00 | ..... |

[a] Difference required for significance (19: 1) between varieties at a station on a given date 0.07.

[b] Difference required for significance (19: 1) between dates at a given station 0.03.

## Canning Quality

In 1939 samples of ungraded whole pods from one of the mid-season pickings of each variety at Green Bay were processed at a commercial

cannery. Samples drawn from the pack were graded by a representative of the Bureau of Agricultural Economics, U. S. Department of Agriculture. All showed a maximum score as to clearness of liquor. All graded within 80 per cent of the maximum for uniformity of color. Each was graded at 95 per cent of the maximum score for flavor. It is thus evident that all the mosaic-resistant varieties tested were equal in canning quality to the mosaic-susceptible Stringless Green Refugee.

## Discussion and Summary

The control of disease through the development of resistant varieties commonly involves the outcrossing of a susceptible variety well-adapted to a certain environment and a commercial need, with a less desirable highly resistant strain. Such was the case in the improvement of Stringless Green Refugee bean for resistance to common mosaic. The present investigation was planned to compare the susceptible variety with 5 resistant varieties as to yield, pod shape, rate of production, and canning quality.

By comparison of the commercial stock of the susceptible variety with one in which the seed was virus-free, it was shown that the disease not only reduces the yield but distinctly alters the production rate. Four of the resistant varieties, Idaho Refugee, U. S. No. 5 Refugee, Sensation Refugee 1066, and Sensation Refugee 1071, were closely similar to the mosaic-free susceptible variety in quantity of yield and rate of production. Sensation Refugee 1066 was consistently earlier than all others in production of pods at the canning stage. Wisconsin Refugee was distinctly later in maturity than the other 4 resistant varieties and usually lower in total yield. Its production increased gradually to a peak late in the harvest period; and in this respect it was similar to the behavior of the susceptible variety when the latter was affected early by mosaic.

No significant differences of importance were found between the resistant varieties and the susceptible one as to pod shape and size. All had equally high canning quality.

All of the desirable characteristics of the original susceptible variety seem to have been retained in the resistant forms. Among the last-mentioned is a range of maturity from that of the earliest, Sensation Refugee 1066 to the latest, Wisconsin Refugee.

A variegation inherited from the resistant parent occurs in Wisconsin Refugee and Idaho Refugee. Its expression is repressed by high air temperatures. Although it did not have a significant influence upon yield, it should be removed from these varieties by selection, since affected plants produce some distorted pods undesirable for processing.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN.
MADISON, WIS.

LITERATURE CITED

1. ANDERSON, M. E. Sensation Refugee, two new mosaic-resistant varieties. Canner 92(7): 14–15. 1941.
2. BURKHOLDER, W. H., and A. S. MULLER. Hereditary abnormalities resembling certain infectious diseases in beans. Phytopath. 16: 731–737. 1926.
3. HARRISON, A. L., and W. H. BURKHOLDER. Canning bean diseases in New York in 1936. U. S. Bur. Plant Ind. Plant Dis. Rptr. 20: 290–291. 1936.
4. HORSFALL, JAMES G., W. H. BUCKHOLDER, and O. A. REINKING. Diseases of Green Refugee beans in New York in 1937. *Ibid.* 21: 318–319. 1937.
5. JOHANNSEN, W. Über Knospenmutation bei Phaseolus. Ztschr. f. Inducktive Abstam. u. Vererbungslehre 1: 1–10. 1909.
6. PARKER, M. C. The inheritance of a yellow-spot character in the bean. Jour. Hered. 24: 481–486. 1933.
7. ————. Inheritance of a leaf variegation in the common bean. *Ibid.* 25: 165–170. 1934.
8. PIERCE, W. H., and J. C. WALKER. The development of mosaic-resistant Refugee beans. Canner 77(26): 7–9. 1933.
9. TJEBBES, K., and H. N. KOOIMAN. Erfelijkheidsonderzoekingen bij Boonen III. Albinisme. Genetica 1: 532–538. 1919. [English summary, pp. 536–538.]
10. WADE, B. L. Genetic studies of variegation in snap beans. Jour. Agr. Res. [U. S.] 63: 661–669. 1941.
11. ————, and W. J. ZAUMEYER. U. S. No. 5 Refugee, a new mosaic-resistant Refugee bean. U. S. Dept. Agr. Circ. 500, 11 pp. 1938.
12. ZAUMEYER, W. J. Inheritance of leaf variegation in beans. Jour. Agr. Res. [U. S.] 64: 119–127. 1942.

# THE RUSTS OF SAFFLOWER[1]

I. L. CONNERS[2,3]

(Accepted for publication March 8, 1943)

## INTRODUCTION

Safflower (*Carthamus tinctorius* L.) has been cultivated from ancient times for the dye extracted from the flowers and the oil present in the seed (7). It is still cultivated extensively in India and Egypt for its oil seed and to a small extent in China, Japan, Turkestan and parts of Europe (16). Since the revolution, safflower evidently has been tested in widely separated regions of the Soviet Union as a source of oil. The plant also has been under trial since 1925 in the United States (16) and still more recently in Canada.

Upon the discovery of *Puccinia carthami* on safflower in Canada, a search was made to determine the history of the rust and its geographical distribution. The scattered nature of the literature appears to warrant the publication of information available on this and the other rusts of safflower.

## PUCCINIA CARTHAMI

*Puccinia carthami* Corda was observed for the first time in Canada in 1942. It was collected on safflower in the variety plots at Saskatoon, Saskatchewan, on September 3, by R. C. Russell (S *1264*) (Dept. Agr. Ottawa Myc. Herb. *12016*). Rust infection was light to moderate on several lines or varieties. Subsequently, it was learned that a similar observation was made by T. C. Vanterpool, who examined the same plots on August 15. The rust, however, was collected as early as July 28 at Morden, Manitoba, by W. E. Sackston (Dept. Agr. Ottawa Myc. Herb. *12017*). The severity of infection on the same plants was estimated by B. Peturson to be 20 per cent on August 26. Uredinia and telia, the only known spore stages for the species, develop in small scattered pulverulent pustules (23, p. 33) and are present in both specimens. The material was found to agree well with the description and figure published by Arthur (1, p. 349). The identification was confirmed by G. B. Cummins, Purdue University, Lafayette, Indiana, who compared part of the Saskatoon collection with the single American collection from Massachusetts, and with three collections from the Old World in the Arthur Herbarium. Portions of the latter were afterwards made available for study. Certain specimens were also kindly

[1] Contribution No. 728 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Associate Plant Pathologist, Central Experimental Farm, Ottawa, Canada.

[3] The author wishes to acknowledge his indebtedness to those who aided him in this study, particularly to Dr. G. B. Cummins for advice and help with the literature, to Dr. M. I. Timonin for translating parts of Tranzschel's work from the Russian, and to Dr. R. C. Russell for supplying the data obtained by him from an examination of samples of safflower seed.

loaned by D. H. Linder from the Farlow Herbarium of Cryptogamic Botany, Harvard University, Cambridge, Mass.

From an examination of seed samples, details of which are reported later, the rust evidently occurred at Brooks, Alberta, in 1939; at Ottawa, at least in 1940; and at Indian Head, Sask., in 1941 and 1942. However, the rust was not observed at Winnipeg, Man., where the safflower plots at the University were examined in September, nor was it found on seed of the 1942 crop from Lethbridge, Alta., or in the plots during a general survey for disease carried out at the Station in the same year.[4]

The occurrence of this rust in Canada is of considerable interest, as it has been reported only once previously in North America, and because safflower may yet become a crop of some importance in Canada and the United States. The sole American record is based on a collection made on *Carthamus tinctorius* at Cottage City, Mass. (2, p. 512), in 1895 (1, p. 349). Although other diseases have been found on the crop during the recent trials in the United States (16), no mention of the rust is made. No additional specimens or records are known to the Division of Mycology and Disease Survey, Bureau of Plant Industry Station, Beltsville, Maryland.[5]

*Puccinia carthami* was described by Corda in 1840 on *Carthamus tinctorius* collected at Prague in Bohemia (2, p. 512). The Sydows (22, 1: 35) record, in 1902, its collection at Ober Glogau in Silesia, at Saaz and Prague in Bohemia, near Giza in Egypt by G. Schweinfurth, and in eastern India and Japan. Two specimens on this host from Japan have been examined: Botanical Gardens, Tokio, Y. Tanaka, June, 1888 (Farlow Herbarium); Sapporo, Prov. Ishikari, Naohide Hiratsuka, July 21, 1926 (Dept. Agr. Ottawa, Myc. Herb. *12018*, ex Herb. Arthur *F8614*). As noted by Tranzschel (23, p. 402), it would appear that the rust occurs widely wherever the safflower is cultivated. He records it on safflower from the following localities in the Soviet Union—Azov-Black Sea region: Rostov, Maikop, Kayal, Armavir, Otrada Kubanskya; Armenia; Azerbaijan: Kirovabad; Uzbekistan: Tashkent (23, p. 372). The rust is known in Transylvania, where it was collected near Cluj, distr. Cojocna, by M. Péterfi, Sept. 4, 1918 (Fl. Roman. *204*, copy in Farlow Herb. examined). It also has been recorded on *Carthamus tinctorius* in India at Pusa and in the Punjab (3, p. 65), in Cyprus (14, p. 15), and again in Egypt (19, p. 130; 11, p. 37). Its occurrence in Italy is based on a single brief mention by Bagnis (24, p. 96), and, although the host is cultivated in different regions of the Iberian peninsula, and is subspontaneous in others (6, pp. 281, 377), it is uncertain whether or not the rust has been collected in Spain.

*Puccinia carthami* has been reported also on: *Carthamus ?creticus* (probably = *C. lanatus* L.) by Bagnis (24, p. 96); *dianius* Sch. (= *Carduncellus caeruleus* (L.) Less.) in Spain (6, p. 378); *C. glaucus* Bieb. at Kyrenia, Cyprus, R. M. Nattrass *810*, May, 1936 (14, p. 15), on the label

[4] Letter from Dr. M. W. Cormack, Edmonton, Alta., Dec. 22, 1942.
[5] Letter from Mr. J. A. Stevenson, Nov. 27, 1942.

of the specimen examined (Mycol. Herb., Ottawa, *12020;* ex Herb. Arthur *F9436*) the host is given, however, as *C. lanatus* L.; *C. lanatus* L. at Canoblas N. S. W. (20, p. 22); *C. oxyacantha* Bieb. at Kangra, Punjab, India, E. J. Butler, May 4, 1905 (21, p. 256), the copy of P. Sydow, Ured. *2116* in the Arthur Herbarium examined (Mycol. Herb., Ottawa, *12019;* ex Herb. Arthur *F8615*); in the Soviet Union in Azerbaijan: Jebrail, Kirovabad, Gheokchay; Turkmeniya: Kopet Dagh (23, p. 372).

A doubtfully distinct rust, *Puccinia kentrophylli* Syd., is reported on *Carthamus lanatus* L. (*Kentrophyllum lanatum* (L.) DC.) in the Soviet Union in Turkmeniya: Ashhabad, Kopet Dagh; Tadjikistan: Gorny Zeravshan (23, p. 372); *Kentrophyllum creticum* (probably = *Carthamus lanatus* L.), and *K. syriacum Boiss.* (= *C. glaucus* Bieb.) in Crete (22, **1**: 104).

The host genus *Kentrophyllum* is no longer considered distinct from *Carthamus.* Accordingly, if *Puccinia kentrophylli* is to be maintained as a species distinct from *P. carthami,* sound morphological differences are required for their separation. To throw some light on this point, the descriptions of the 2 species as they are given by the Sydows (22, **1**: 104 and 35) and Arthur and Mains (2, p. 512) were compared with the rust in the 7 collections studied. As a result of the comparison, there appeared to be no sound basis for their separation. Sections of the sori disclosed uredia, with teliospores entirely absent, and telia, containing at least some urediospores. In one small unbroken sorus, several urediospores were present, but teliospores were already beginning to form. It is suggested that the possible absence of uredia in the original material, from which *P. kentrophylli* was described, was due to the collection being scanty or because, with the advance of the season, only telia were being formed on the green parts of the plant as a result of secondary infection. The telia of *P. kentrophylli* are given as pulverulent, but remaining covered by the epidermis. Generally, the pustules are soon naked, but the ruptured epidermis is usually evident, and some sori are still covered. The pedicel of the teliospore in *P. kentrophylli* is said to be hyaline, slender, up to 50 μ in length, but deciduous, while the teliospore pedicel in *P. carthami* is reported to be hyaline and very short by the Sydows in contrast to colorless, delicate, fragile, mostly deciduous by Arthur and Mains. In sections of the sori, the pedicel was found to be variable in length and may reach 65 μ or more. It is very delicate and soon collapses, except for a short portion, 3–7 μ in length, adjacent to the spore. This piece, usually with only a small part of the collapsed pedicel adhering, is detached with the spore. The basal portions persist, however, after the spores have disappeared and are not to be confused with paraphyses, for, in young sori, no free hyaline elements are present. Spores in all stages of development, with the younger nearer the floor of the sorus, may be seen crowding through them. The urediospore pedicels similarly persist after the spores have become detached. Considerable variability was observed in the size of the teliospores, the pigmentation and markings of the spore wall, etc., from collection to collection,

but these characters did not seem to be correlated in any way. It may be noted that the wall of the urediospore is often thicker at the point of attachment to the pedicel than elsewhere. In all the collections, 3, or occasionally 4, equatorial pores are present. Contrary to later observers, but 2 pores are reported by the Sydows for *P. carthami.* The correctness of their statement can only be settled by a re-examination of the older European collections available to them.

A closely related species is *Puccinia carduncelli* Syd. reported on *Carduncellus caeruleus* (L.) Less. in Sicily (22, 1: 33). This rust was studied from a specimen on the above host from Orleansville, Algeria, L. Trabut, June 20, 1912 (R. Maire, Myc. Bor.-Africa. *86,* copy in Farlow Herb.). Only telia were found. The teliospores resemble closely those of *P. carthami,* but are remarkable for their great variability in size and shape. A few urediospores were also located. They were ovate, rather than ellipsoid, with two superequatorial germ pores placed opposite each other. The rust is apparently the same as one described by Malençon[6] from specimens collected on *Carthamus calvus* (Boiss. and Reut.) Bott. at Dayet Ahoua (Moyen-Atlas) June 19, and Oujda on June 24, 1936, in Morocco, but referred by him to *P. carthami.* Maire and Werner[7] transferred the rust to *P. carduncelli.* There is a suggestion that Boissier and Reuter were correct when they originally described the host as *Carduncellus calvus.* It is very probable that the rust reported on *Carthamus dianius* (= *Carduncellus caeruleus*) from Spain (6, p. 377) belongs here rather than in *P. carthami.*

### OCCURRENCE OF TELIOSPORES OF PUCCINIA CARTHAMI ON SAFFLOWER SEED

As safflower is a cultivated, rarely adventive plant in several countries of the world, including Canada and the United States, the scattered appearance of *Puccinia carthami* in these countries must be due to its chance introduction as suggested by the Sydows (22, 1: 36). In Europe, there is the possibility that a rust on some native host might have spread to safflower, but such a possibility seems very remote in North America. It would appear more probable that spores carried on the seed might cause infection of the plant. To determine whether spores were present or not on safflower seed, 14 samples of seed were obtained from W. G. McGregor, Cereal Division, Central Experimental Farm, Ottawa. A test tube was filled with about 1½ inches of seed, water was added to fill about 3 inches of the tube. The tube was then shaken vigorously. The washings, about 10 cc., were pipetted off and centrifuged. The supernatant liquid was decanted off. The sediment was shaken in 2–3 drops of water and poured out on a slide. The latter was gently heated to drive off most of the water, 2 drops of lactophenol were added and the slide heated further to clear the spores.

[6] Malençon, G. Notulae mycologicae maroccanae, II. Rev. Mycol. 1–2: 263. 1936.
[7] Maire, R., and R. G. Werner. Fungi maroccani. Catalogue raisonné des champignons connus jusqu'ici au Maroc. Mem. Soc. Sci. Maroc. 45, p. 62. 1937 (1938).

TABLE 1.—*Occurrence of teliospores of Puccinia carthami on safflower seed examined at Ottawa*

| Designation | Place of origin | Place sample grown | Year | Number of spores |
|---|---|---|---|---|
| Type 1 | India | Lambeth, Mont. | ..... | 0 |
| Type 7 | " | " " | ..... | 0 |
| Type 14 | Russia | " " | ..... | 3 |
| Type 25 | Pusa, India | " " | ..... | 0 |
| C.D. 2651 | United Provinces, India | India | ..... | 0 |
| C.D. 3230 | Hungary | Brooks, Alta.[a] | ..... | 2,500[b] |
| C.D. 3230 | " | Ottawa | 1940 | 27, 39[c] |
| C.D. 3230 | " | " | 1941 | 0, 0[c] |
| C.D. 3230 | " | " | 1942 | 0, 8, 1[c] |
| Type 1 | | " | 1942 | 0 |
| Type 6 | Saratov, Russia | " | 1942 | 0 |
| Type 14 | | " | 1942 | 0 |
| Type 25 | | " | 1942 | 0 |
| C.D. 2650 | United Provinces, India | " | 1942 | 0 |

[a] Seed from D. Demetrovits, Brooks, Alta., April 15, 1940.
[b] Estimated number. An average of 21 spores was counted in each of 6 low-power fields. Urediospores were also abundant.
[c] Duplicate or triplicate examinations.

As Table 1 shows, the seed received from Brooks, Alta., was carrying a heavy spore load. It is believed that the original seed was imported from Hungary in 1939 and was grown at Brooks the same year. Some rust developed in the plots at Ottawa in 1940, with possibly small amounts in 1941 and 1942. Later, 10 samples of seed grown at Lethbridge, Alta., in 1942, and furnished by W. D. Hay were examined, but no teliospores that could be referred with certainty to *P. carthami* were seen.

In addition, 29 samples were examined by Dr. Russell, who used a slightly different technique, but his figures may be safely compared with mine, if the former are doubled.

TABLE 2.—*Occurrence of teliospores of Puccinia carthami on seed examined at Saskatoon, Sask.*

| Number of samples | Place grown | Year grown | Number of spores | |
|---|---|---|---|---|
| | | | Range | Average |
| 5 | Indian Head | 1941 | 12–25 | 19.2 |
| 5 | Ottawa | " | 0–1 | 0.2 |
| 3 | Lethbridge | " | 0 | 0.0 |
| 3 | Indian Head | 1942 | 2–5 | 3.3 |
| 9 | Saskatoon (variety plots) | " | 78–233 | 137.1 |
| 3 | Saskatoon (increase plots) | " | 0–13 | 5.2 |
| 1 | Sutherland | " | 0 | 0.0 |

The results presented in Table 2 indicate that rust infection was greater in the variety plots at Saskatoon than at any other point from which seed

was examined by Russell. The infection was known to be light to moderate in these plots in September. By comparison of the spore loads recorded in the two tables, it may be inferred that the heaviest infection so far encountered occurred at Brooks, Alta. However, when this seed was planted at Ottawa in 1940, it yielded a crop but lightly infected. This fact suggests that seasonal conditions play an important part in rust development. If the appearance of this rust in Canada is due to infection from spores carried on the seed, it seems probable that the rust reached Ottawa from Brooks, and has spread from Ottawa to the other points mentioned. This is a reasonable assumption, for the Cereal Division at Ottawa has supplied, directly or indirectly, most of the lines under test in Western Canada, both to points where rust has been found and also to those where it has not been detected. Although the rust has never been reported to be destructive, it is impossible to hazard a guess concerning its importance to safflower culture until something is known of its epidemiology. Varietal differences in rust susceptibility are also to be expected.

### PUCCINIA VERRUCA

A second, quite different rust occurs on safflower. It forms compact telia aggregated into relatively large groups. On safflower it has been recorded only within the boundaries of the Soviet Union (23, p. 33) in fields of the plant at Odessa, Rostov, and Omsk. On this host it originally was described as *Puccinia jaczewskii* Tropova, Journ. of Agric. Research North Caucasus **5** (22): 211. fig. 1930 (23, p. 387). Under the name "*Puccinia centaureae,*" Mourashkinsky (12) reported it as a minor disease of safflower at Omsk, where it was very prevalent on *Centaurea scabiosa* L. Later, he (13) showed by morphological studies and cross-inoculation experiments that the rust on safflower was identical with *Puccinia verruca* Thüm. (cfr. 22, 1: 42), which is recorded on many species of Centaurea in Europe, Asia, and North Africa but does not occur in North America. Accordingly, *Puccinia jaczewskii* may be considered a synonym of *Puccinia verruca.* It is also quite probable that *Puccinia sommieriana* Sacc. (18, p. 560) described on *Centrophyllum lanatum* (= *Carthamus lanatus*) from Malta is another synonym. A specimen of *P. sommieriana* on *Kentrophyllum lanatum,* obtained at Vias, Hérault, France, by De Crozals, June, 1913. Comm. P. Hariot (Vesterg. Micr. Rar. Sel. *1719,* Herb. Arthur *F8261*) was compared with one of *P. verruca* on *Centaurea napifolia* L. (D. Sacc. Myc. Ital. *707*) and no distinguishing features were observed.

*Puccinia verruca* is a microcyclic species. The teliospores are narrow, smooth, usually thickened at the apex and with a long persistent pedicel. Except that the spores were less highly pigmented, they suggest those of *Puccina asteris* Duby, a microcyclic species on *Aster.* The similarity of the two species has already been noted by the Sydows (22, 1: 43).

The close morphological resemblance between the telia of micro species and those of long-cycled heteroecious species has led to the correlation of

the respective species (8). Dietel (4, p. 492) has already pointed out the correlation of *Puccinia verruca* with its III on *Centaurea,* and now also on *Carthamus,* with *Puccinia centaureae-caricis* Tranz., which has its II, III on *Carex* and I on *Centaurea.* According to Klebahn (9, p. 516) *Puccinia centaureae-caricis* was proposed by Tranzschel in 1909 as a collective species. Klebahn reduced the *Centaurea-Carex* species of *Puccinia,* which had been described up to that time, to the rank of special forms, and united them under the above name. However, if the present rules of botanical nomenclature are followed, this rust should be known as *Puccinia arenariicola* Plowr. (Journ. Linn. Soc. **24**: 90. Aug. 20, 1887).[8] The teliospores of *Puccinia verruca* are in general narrower and less highly colored than given for any of the segregates of *P. centaureae-caricis,* but they approach most closely the dimensions given in the original description of *P. tenuistipes* Rostr., as set down by Klebahn (9, p. 518). The Sydows (22, **1**: 43) have noted that the teliospores of *P. verruca* are very variable.

### AECIDIUM CARTHAMI

The third rust reported on safflower is a rare aecium, *Aecidium carthami* Dietr. (5, p. 284) collected by him at Heimar in Ehstland and reported by Rodighin (17) in the Soviet Union in the Saratov district. However, Lepik (10) recently examined the specimen issued by Dietrich (Plant. fl. balt. crypt. Cent. VIII, No. *20*) and reported that *A. carthami* is an aecium on an unidentifiable host plant. According to Curator K. Eichvald, the host may be a species of *Centaurea,* but under no circumstances is it *Carthamus tinctorius.* Even if *Aecidium carthami* was founded on an error, there is no reason to doubt that Rodighin collected an aecium on safflower. Tranzschel (23, p. 382) suggests such an aecium is possibly the result of infection by *Puccinia centaureae-caracis.* This seems a reasonable suggestion, as the correlated microcyclic species, *P. verruca,* has been shown to occur on *Carthamus.* Finally, it should be noted that Oudemans (15, **4**: 1058) is quite incorrect in listing *Aecidium carthami* as a synonym of *Puccinia carthami;* particularly, as this error appears again in the abstract of Rodighin's paper in the Review of Applied Mycology (17), where the name is written *Aecidium* [*Puccinia*] *carthami.*

### SUMMARY

1. The rusts, *Puccinia carthami, P. verruca* and *Aecidium carthami,* have been reported on safflower.

2. *Puccinia carthami* is reported for the first time in Canada. It was collected at Morden, Man., and Saskatoon, Sask., in 1942.

3. The rust probably occurred at other points in Canada, as teliospores of the rust were found on seed samples from these points.

4. *Puccina kentrophylli,* which has been reported on some species of *Carthamus,* is not considered to be distinct from *P. carthami.* On the other

[8] Cfr. Barnhart, J. H. Bibliography. N. Am. Flora 7: 1083. 1940.

hand, *P. carduncelli* is apparently a well-defined species, which has been reported on *Carduncellus caeruleus* and *Carthamus calvus*.

DIVISION OF BOTANY AND PLANT PATHOLOGY,
CENTRAL EXPERIMENTAL FARM,
OTTAWA, CANADA.

LITERATURE CITED

1. ARTHUR, J. C. Manual of the rusts in United States and Canada. pp. i–xv. 1–438. Lafayette. 1934.
2. ———— and E. B. MAINS. Bullaria. N. Am. Flora 7: 482–515. 1922.
3. BUTLER, E. J., and G. R. BISBY. The fungi of India. pp. i–xviii, 1–237. Calcutta. 1931.
4. DIETEL, P. Über die wirtswechselnden Rostpilze. Centralbl. f. Bakt. Abt. 2. 48: 470–500. 1918.
5. DIETRICH, H. A. Blicke in die Cryptogamenwelt der Ostseeprovinzen. Archiv. f. Nat. Liv-, Ehst- u. Kurlands. Ser. 2. 1: 261–414. 1856.
6. GONZÁLEZ, FRAGOSO R. Flora Iberica. Uredales. Tomo I. Genero Puccinia. pp. i–lxxi, 1–146. Madrid. 1924.
7. HEGI, G. *Carthamus tinctorius* L. *In* Illustrierte Flora von Mittel-Europa 6(2): 986–989. München.
8. JACKSON, H. S. Present evolutionary tendencies and the origin of life cycles in the Uredinales. Mem. Torrey Bot. Club 18: 1–108. 1931.
9. KLEBAHN, H. Uredineen. Kryptogamenflora der Mark Brandenburg 5a: 67–904. 1912–1914.
10. LEPIK, E. Beiträge zur Nomenclatur der ostbaltischen Pilzflora IV–VI. IV. Revision der "Plantarum florae balticae cryptogamarum." Collegit et edidit A. H. Dietrich, Uredinales und Ustilaginales aus Centurien I–IX, Revaliae MDCCCLII–MDCCCLVII. Ann. Soc. Rebus Nat. Invest. Univ. Tartu. Constitutae 45: 1–80. 1938 (1940).
11. MELCHERS, L. E. A check list of plant diseases and fungi occurring in Egypt. Trans. Kans. Acad. Sci. 34: 41–106. 1931.
12. MOURASHKINSKY, K. E. [Diseases of the safflower]. Reprinted from [Bull. West Siberian Sect., Russian Geogr. Soc.] 5: 6 pp. 1926. (in Russian). Abstract in Rev. Appl. Mycol. 6: 354–355. 1928.
13. ————. [New diseases of cultivated plants in Western Siberia]. Trans. Omsk. Inst. Agric. 1(6): 3–30. 1935. (in Russian with English summary). Abstract in Rev. Appl. Mycol. 14: 493–494. 1936.
14. NATTRASS, R. M. A first list of Cyprus fungi. pp. v–xvi, 1–87. Nicosia. 1937.
15. OUDEMANS, C. A. J. A. Enumeratio systematica fungorum.1–5: Hagae. 1919–1924.
16. RABAK, F. Safflower, a possible new oil-seed crop for the northern Great Plains and the far Western States. U.S.D.A. Circ. 366: 1–14. 1935.
17. RODIGHIN, M. N. [Rare and little known fungous diseases of safflower in the Volga region]. "25 years Saratoff Agricultural Institute" pp. 186–190. Saratoff. 1939. (in Russian). Abstract in Rev. Appl. Mycol. 19: 115–116. 1940.
18. SACCARDO, P. A. Notae mycologicae. Series XVII. Ann. Mycol. 11: 546–568. 1913.
19. SNELL, K. Beiträge zur Kenntnis der pilzparasitären Krankheiten von Kulturpflanzen in Ägypten und ihrer Bekämpfung. Angewandte Bot. 5: 121–131. 1923. Also abstract in Rev. Appl. Mycol. 3: 323–324. 1924.
20. SYDOW, H. Neue oder bemerkenswerte australische Micromyceten—I. Ann. Mycol. 35: 22–49. 1937.
21. SYDOW, H. and P., and E. J. BUTLER. Fungi Indiae orientalis. Pars IV. Ann. Mycol. 10: 243–280. 1912.
22. SYDOW, P. and H. Mongraphia Uredinearum. 1–4. Lipsiae. 1902–1924.
23. TRANZSCHEL, W. Conspectus Uredinalium URSS. pp. 1–426. Mosqua (in Russian). 1939.
24. TROTTER, A. Flora Italica Cryptogama, Uredinales. pp. 1–519. Rocca S. Casciano. 1908–1914.

# TOMATO SEED TREATMENTS IN RELATION TO CONTROL OF ALTERNARIA SOLANI[1]

W. D. Moore, H. Rex Thomas, and Edward K. Vaughan

(Accepted for publication February 16, 1943)

## INTRODUCTION

The area in the South devoted to growing tomato seedlings for the commercial trade has increased from an initial small acreage about 20 years ago to a total of approximately 8000 acres in 1942. The major portion of this acreage is located in southern Georgia, although important plantings are made in Florida, Mississippi, Tennessee, and Arkansas. Occasional serious losses from epiphytotics of both fungus and bacterial diseases have been one of the most serious handicaps that have confronted this expanding industry. Of the several maladies affecting tomato seedlings in the South, the one caused by *Alternaria solani* (Ell. and Mart.) Jones and Grout is the most common and, in the majority of instances, the most destructive. The losses caused by this organism result partly from damage to and a limited mortality of seedlings in the plant bed immediately after germination but, more commonly, from foliage and stem infections that cause poor stands and weakened plants in the commercial fields following transplanting. Since some part of all nontreated commercial tomato seed is known to carry a limited amount of *Alternaria solani* inoculum, the value of seed treatment as a means of control for this disease is of considerable economic interest.

## REVIEW OF LITERATURE

Bichloride of mercury has long been recognized as a safe and effective chemical for the treatment of many kinds of vegetable seed, including that of the tomato. While this material is an excellent surface disinfectant, it has the disadvantage of leaving the seed open to recontamination after treatment (11). This is of particular importance where treated seeds are dried in rooms in which the air is apt to be loaded with parasitic organisms. Samson (12) and Vaughan[2] have shown that some of the organic mercury compounds have sterilizing potentialities equally as high as bichloride of mercury and, in addition, leave a residue of the active disinfectant material on the seed coat, which protects it against subsequent recontamination. Extensive tests are reported by Clayton (1, 2), Horsfall (3, 4, 5), and Moore *et al.* (9) in which these and other materials were employed as seed treatments on various vegetable seeds. Aside from that portion of the damping-

[1] Conducted as a phase of cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture and the Department of Botany, Indiana Agricultural Experiment Station; the New Jersey Agricultural Experiment Station; the Georgia Coastal Plain Experiment Station; the Georgia Agricultural Experiment Station; and the Georgia State Department of Entomology.

[2] Vaughan, E. K. The use of ethyl mercury phosphate for treating tomato seed in New Jersey.

off that may have been caused by *Alternaria solani,* no reference is made in any of these reports to the control of the disease on tomato seedlings at later stages of growth. That such treatments, however, may be beneficial in the control of this fungus in seedling fields is suggested by Miller (7), Miller and Crosier (8), and Van Haltern (15, 16).

Few if any workers have doubted the possibility of tomato seed being the source of considerable dissemination of *Alternaria solani.* Massie (6) demonstrated this as early as 1914. However, the fact that in the South[3] well-isolated fields of new soil, when planted to seed known to be thoroughly disinfected, have frequently shown infections by *Alternaria solani,* raised several questions not previously explained. Since a limited amount of the organism has been found internally in some lots of tomato seed, it has been suggested that this would account for the presence of the disease in fields of virgin soil planted with properly disinfected seed. Following some carefully conducted experiments on this phase of the seed problem, Samson (12) reports: "it appears unlikely that commercial seed may include any significant proportion internally infected with *Macrosporium solani.*" Later work by Samson, Nugent, and Shenberger (14) further verifies this conclusion. In approaching the whole problem of *Alternaria solani* control, whether by seed treatment or other means, a consideration of an existing widespread dissemination of this organism in the soil throughout the plant producing area of the South is of considerable importance. Significant evidence in support of this premise is given in another report by the writers.[3] That widespread dissemination of this organism occurs in other areas is indicated by Samson and Thomas (13) who report that "*early blight* fungus is widespread, occurring naturally in all parts of Indiana, and is undoubtedly present on all forms, regardless of previous tomato crops." In order to secure additional information on the seed-treatment problem, in so far as it applies to the tomato-seedling industry in the South, a series of experiments was undertaken in 1937 and continued, with the exception of 1940, through the season of 1942. Some tests also were conducted in Indiana and New Jersey.

## PROCEDURE

Commercial lots of nontreated tomato seed, known to be infected with *Alternaria solani,* were used in all experiments. All chemicals used for treatments were tested in preliminary laboratory and greenhouse tests to study their disinfectant properties and subsequent influence on germination. Effectiveness against both bacteria and fungi was determined by plating samples of treated and nontreated seed on potato-dextrose agar and incubating for 5 days at 25° C. After thus investigating many possible seed-treatment materials, 9 were chosen for critical tests under field condi-

[3] More, W. D., H. Rex Thomas, and E. K. Vaughan. Aerial dissemination of the *Alternaria solani* spores, and its possible influence upon field infection of tomato seedlings. (Unpublished manuscript.)

tions. As it became evident that certain treatments were relatively ineffective or undesirable, this number was further lowered from season to season. In 1942 only such treatments were applied as apparently gave 100 per cent surface sterilization of the seed, hence the nontreated check lots were the only seeds that carried surface contamination.

The experimental plots were located each season on land known to have been free of solanaceous crops for 10 years or more and also were well-isolated from such crops during the period of the experiments. A Latin-square design was used in all experiments, except those of 1942. The individual plot rows were 16 inches apart and 50 feet in length. The seeding rate was 20 seeds per foot. In 1942, the treatments were planted in blocks 20 feet × 22 feet, 14 rows per block, with separation alleys 20 feet wide between blocks. All treatments in this experiment were replicated 3 times. Fertilizer applications, depth of planting, and cultivation conformed to local commercial practice. Germination readings were made as soon as a commercially satisfactory stand was shown by one or more of the several treatments. Disease readings were confined to one observation, this being taken as soon as the plants reached the commercial shipping size of 6 to 10 inches high. In all cases this size was attained within 40 to 55 days after seeding. Initial leaf-spot infection was evidenced by small, dark-brown spots on the lower leaves, while stem canker appeared as small, irregular, dark-colored lesions along the main stems and leaf petioles. When the plants reached the shipping size, random samples of 50 were pulled from each replicate of the several treatments and the number of plants showing infection noted. At the same time, the total number of stem cankers per replicate was recorded. In the 1942 experiment all plants of shipping size were pulled on all plots when leaf spot first appeared.

### INFLUENCE OF SEED TREATMENT ON GERMINATION

One of the most important considerations in selecting any seed-treatment material is its effect upon germination. It is particularly important to consider this in connection with Southern tomato plant production, since the seeds are planted during early spring when the weather may vary from cold rains to warm drying winds. A seed-treatment material that has a markedly retarding effect upon germination under either of these conditions is undesirable, regardless of its effectiveness in other respects. In table 1 are data showing the influence of several treatments, replicated 6 times, on seed germination when planted in sand in the greenhouse during periods of high temperature and medium humidity. Under these conditions it appears that no treatment increased stands significantly, while marked and significant decreases were shown in several instances. This was particularly true where combinations of both liquid and dust treatments were used. While the soil and air conditions during the course of these tests were admittedly not comparable to average field conditions during the spring months, the reaction

TABLE 1.—*The influence of tomato seed treatments on stands in greenhouse experiments conducted at New Brunswick, N. J., 1941*

| Number | Treatments | Mean stands | |
|---|---|---|---|
| | | Exp. No. 1 | Exp. No. 2 |
| 1 | Nontreated check | 40.3 | 41.8 |
| 2 | $HgCl_2$ (1–3000) for 10 min. | 15.1 | 36.1 |
| 3 | Hot water, 55° C. for 10 min. | 43.1 | 44.8 |
| 4 | New Improved Ceresan[a] (1–1200) for 5 min. | 25.1 | 36.8 |
| 5 | 1% ethyl mercury tartrate (1–100) for 5 min. | 24.8 | 34.1 |
| 6 | Malachite green (1–2000) for 5 min. | 28.1 | 36.1 |
| 7 | New Improved Ceresan[a] (1–1200) for 5 min. + Cuprocide dust | 15.0 | 27.8 |
| 8 | Cuprocide dust[b] | 26.5 | 39.3 |
| 9 | New Improved Ceresan[a] (1–1200) for 5 min. + Vasco-4 dust | 21.8 | 36.3 |
| | Required difference for significance | 8.2 | 8.6 |

[a] 5% ethyl mercury phosphate.
[b] Cuprous oxide.

of the various treated seed lots is of considerable value in interpreting other data collected during the course of these studies.

Field data for the same treatments in trials conducted in Georgia, New Jersey, and Indiana during 1941 are shown in table 2. Rainfall and tem-

TABLE 2.—*The influence of tomato seed treatments on stands in field experiments conducted during 1941 at Tifton, Ga., Lafayette, Ind., and Riverton, N. J.*

| Number | Treatments | Mean stand per plot | | |
|---|---|---|---|---|
| | | Ga. | Ind. | N. J. |
| 1 | Nontreated check | 63.5 | 57.2 | 39.2 |
| 2 | $HgCl_2$ (1–3000) for 10 min. | 64.2 | 53.6 | 76.1 |
| 3 | Hot water, 55° C. for 10 min. | 59.6 | 60.3 | 50.6 |
| 4 | New Improved Ceresan (1–1200) for 5 min. | 63.8 | 45.2 | 68.6 |
| 5 | 1% ethyl mercury tartrate (1–100) for 5 min. | 64.3 | 50.7 | 69.3 |
| 6 | Malachite green (1–2000) for 5 min. | 61.9 | 59.7 | 84.5 |
| 7 | New Improved Ceresan (1–1200) for 5 min. plus Cuprocide dust | 63.8 | 42.7 | 47.0 |
| 8 | Cuprocide dust | 55.7 | 57.4 | 80.2 |
| 9 | New Improved Ceresan (1–1200) for 5 min. plus Vasco-4 dust | 60.0 | 48.7 | 76.6 |
| | Required difference for significance | Not significant[a] | 5.3 | 12.0 |

[a] $F = 0.41$. Required value at $5\% = 2.52$.

peratures in Georgia and Indiana were sufficient for germination, whereas in New Jersey, rain was heavy and temperatures comparatively low. Accordingly, only a few treatments suppressed germination significantly below the nontreated check, and several appreciably increased stands. Other germination data of a similar nature collected in seasons of light rainfall and moderate temperature are given in tables 4, 5, and 6. In the New Jersey experiment of 1941 the seed lots were planted in soils that were fairly cold

and wet. From the data presented it is apparent that under those conditions seed treatments increased germination significantly in several instances. That such reactions may be associated with cold, rainy seasons is suggested by additional stand data (table 3) taken in Georgia in 1937, when the soil was cool and moist during the period of germination.

TABLE 3.—*The influence of tomato seed treatments on stands and on the control of Alternaria solani leaf-spot infection at Tifton, Ga., in 1937*

| Number | Treatments | Mean stand per plot | Mean number leaf spots per plot sample[a] |
|---|---|---|---|
| 1 | Nontreated check | 47.3 | 57.3 |
| 2 | Acidulated $HgCl_2$ (1–3000) for 5 min. | 60.4 | 53.3 |
| 3 | $HgCl_2$ (1–3000) for 5 min. | 62.3 | 41.1 |
| 4 | Formaldehyde (1–200 at 120° F.) for 5 min. | 50.8 | 53.3 |
| 5 | Hot water, 55° C. for 10 min. | 55.2 | 50.7 |
| 6 | Sodium hypochlorite (1–10) for 5 min. | 50.9 | 43.4 |
| 7 | Semesan Jr. (1–160) for 5 min. | 77.2 | 49.7 |
| 8 | New Improved Ceresan (1–800) for 5 min. | 76.4 | 42.1 |
| 9 | 5% ethyl mercury iodide (1–800) for 5 min. | 61.6 | 46.0 |
| 10 | 1% ethyl mercury tartrate (1–160) for 5 min. | 72.7 | 40.3 |
| | Required difference for significance | 16.6 | Not significant[b] |

[a] No stem cankers.
[b] F = 1.77. Required value at 5% = 2.00.

TABLE 4.—*The influence of tomato seed treatment on stands and on the control of Alternaria solani leaf-spot and stem-canker infections at Tifton, Ga., in 1938*

| Number | Treatments | Mean stand per plot | Mean number leaf spots per plot sample | Mean number stem cankers per plot sample |
|---|---|---|---|---|
| 1 | Untreated check | 117.0 | 67.3 | 36.0 |
| 2 | $HgCl_2$ (1–3000) for 10 min. | 72.5 | 82.8 | 21.4 |
| 3 | Hot water, 55° C. for 10 min. | 101.8 | 80.9 | 33.1 |
| 4 | New Improved Ceresan (1–1200) for 5 min. | 75.3 | 82.0 | 25.2 |
| 5 | 1% ethyl mercury tartrate (1–100) for 5 min. | 100.2 | 87.1 | 27.7 |
| 6 | Malachite green (1–200) for 5 min. | 76.1 | 72.4 | 34.0 |
| 7 | New Improved Ceresan (1–1200) for 5 min. plus Cuprocide dust | 83.1 | 79.8 | 29.6 |
| 8 | Cuprocide dust | 97.6 | 77.9 | 26.9 |
| 9 | New Improved Ceresan (1–1200) for 5 min. plus Vasco-4 dust | 56.0 | 78.0 | 17.5 |
| | Required difference for significance | 4.0 | Not significant[a] | Not significant[b] |

[a] F = 1.3. Required value at 5% = 2.07.
[b] F = 1.0. Required value at 5% = 2.07.

## INFLUENCE OF SEED TREATMENT ON LEAF SPOT AND STEM CANKER

Since the development of *Alternaria solani* in either the leaf-spot or the stem-canker stage is markedly limited by the prevailing humidity (10),

wide variation in amount of disease may be expected from one season to another. This was true in the present investigation, where the disease was entirely absent in one season, was present in only the leaf spot stage in two, and caused both leaf spot and stem canker in the others. Due to such variation in seasonal disease development, it was necessary to continue the work

TABLE 5.—*The influence of tomato seed treatment on stands and on the control of Alternaria solani stem-canker infection at Lafayette, Ind., in 1938*

| Number | Treatments | Mean stand per plot | Mean number stem cankers per plot sample |
|---|---|---|---|
| 1 | Nontreated check | 49.2 | 2.1 |
| 2 | $HgCl_2$ (1–3000) for 10 min. | 45.0 | 2.0 |
| 3 | Hot water, 55° C. for 10 min. | 46.6 | 0.8 |
| 4 | New Improved Ceresan (1–1200) for 5 min. | 51.0 | 0.9 |
| 5 | 1% ethyl mercury tartrate (1–100) for 5 min. | 43.8 | 1.4 |
| 6 | Malachite green (1–2000) for 5 min. | 41.6 | 1.8 |
| 7 | New Improved Ceresan (1–1200) for 5 min. + Cuprocide dust | 55.8 | 1.4 |
| 8 | Cuprocide dust | 52.8 | 1.9 |
| 9 | New Improved Ceresan (1–1200) for 5 min. + Vasco-4 dust | 52.0 | 1.9 |
| | Required difference for significance | 6.0 | Not significant[a] |

[a] F = 1.3. Required value at 5% = 1.99.

over a period of 6 years in order to collect sufficient data to warrant definite conclusions as to the value of the seed treatments for control of *Alternaria solani*. From the leaf-spot and stem-canker data presented in tables 3, 4, 5, 6, and 7, it is apparent that in only one instance (1939), was infection by

TABLE 6.—*The influence of tomato seed treatment on stand and on the control of Alternaria solani leaf spot at Tifton, Ga., in 1939*

| Number | Treatments | Mean stand per plot | Mean number leaf spots per plot sample |
|---|---|---|---|
| 1 | $HgCl_2$ (1–3000) for 10 min. | 242.4 | 37.2 |
| 2 | New Improved Ceresan (1–1200) for 5 min. | 238.0 | 31.6 |
| 3 | New Improved Ceresan (1–1200) for 5 min. + Cuprocide dust | 187.0 | 22.4 |
| 4 | New Improved Ceresan (1–1200) for 5 min. + Vasco-4 dust | 243.0 | 30.4 |
| 5 | Check | 346.0 | 40.4 |
| | Required difference for significance | 8.3 | 11.0 |

*Alternaria solani* reduced significantly by seed treatments. In this instance (treatment 3, table 6) germination was delayed. Consequently, the less advanced age and maturity of the plants appeared to be the determining factor rather than the surface sterilization of the seed. This relationship of seedling age to infection by *Alternaria solani* has been demonstrated

TABLE 7.—*The influence of tomato seed treatments on the control of Alternaria solani leaf-spot infection at Tifton, Ga., in 1942*

| Number | Treatments | Mean number leaf spots per plot sample |
|---|---|---|
| 1 | Nontreated check | 77.4 |
| 2 | $HgCl_2$ (1–3000) for 10 min. | 80.6 |
| 3 | New Improved Ceresan (1–1200) for 5 min. | 81.5 |
| 4 | Malachite green (1–2000) for 5 min. | 80.6 |
| 5 | New Improved Ceresan (1–1200) plus Cuprocide dust | 84.1 |
| | | Not significant[a] |

[a] F = 0.22. Required value at 5% = 2.82.

under southern field conditions in other experiments by two of the writers.[4] In nearly all instances, the amount of infection increased with the increasing age of the seedlings.

## DISCUSSION

The influence of tomato-seed treatments on germination and subsequent stands apparently is determined by prevailing weather conditions. During periods of medium to high temperature and moderate to low rainfall the liquid organic mercury treatments and the copper dust treatments have a tendency to depress germination to a point somewhat lower than that of the nontreated seed. During periods of cool wet weather, however, significant increases in stands occurred after both the liquid and the dust treatments. While there were instances where field stands were reduced by certain treatments, the reductions in most cases were not of appreciable importance under the conditions encountered by the writers. Since significant increases were had during cool wet seasons, the practice of seed treatment, if only for protection against damping-off, is highly advisable.

The failure of seed treatments to control *Alternaria solani* on tomato seedlings in the field, under the conditions of these experiments, is due apparently to a wide occurrence of the organism in the soil throughout the plant growing area and not to a lack of thorough surface sterilization of the seed. With a combined annual seedling and market tomato planting of 12,000 to 15,000 acres in southern Georgia, there is ample opportunity for wide distribution of inoculum. Even though there are available several materials that will effectively disinfect tomato seed, there is likely to be a sufficient amount of inoculum already present in all commercial fields, whether old or new, to insure heavy infection under proper weather conditions.[5] This apparently precludes the possibility of a general control of this disease by means of seed treatment alone. While the results of these experiments are not in agreement with the findings of some other investigators (7, 8, 14, 15),

[4] Moore, W. D., and H. Rex Thomas. Some cultural practices that influence the development of *Alternaria solani*. (Unpublished manuscript.)

[5] See footnote 3.

the differences in results appear to be due to differences in circumstances or prevailing conditions rather than in crop technique. In areas outside of the tomato-growing district of Georgia the present data may not apply. Within the restricted commercial areas, however, the above findings have been further verified as to commercial practice from the plant certification records of the Georgia Department of Entomology. From hundreds of cases over a period of 3 years, wherein records were made of seed infection, crop history of the fields, spraying, and disease incidence, no consistent differences were observed in *Alternaria solani* infection between fields planted with treated seed and those planted to nontreated seed. The lack of control of this particular organism in these experiments, however, does not minimize the importance of seed treatment to the tomato-plant industry. The necessity for control of *bacterial leaf spot* and protection against damping-off fully justifies the practice.

## SUMMARY

Both liquid and dust seed treatments have a tendency to retard tomato seed germination during periods of high temperatures and medium to low rainfall. During the two seasons of cool wet weather experienced in the course of this research, final germination was improved significantly by the organic mercury treatments. Bichloride of mercury also gave significant improvement in one of these seasons, and malachite green and Cuprocide dust showed effectiveness during the one season in which they were included.

With the exception of one treatment (Ceresan plus Cuprocide) in 1939, seed treatments did not reduce *Alternaria solani* leaf spot or stem canker significantly in experiments conducted in four different years. Since the treatment mentioned caused no reduction in infection when tested in other years, it appears that the treatments here described are not likely to greatly reduce the amount of *Alternaria solani* infection in the tomato-plant-growing sections of southern Georgia.

AGRICULTURAL EXPERIMENT STATION,
TIFTON, GA.

## LITERATURE CITED

1. CLAYTON, E. E. Vegetable seed treatment with special reference to the use of hot water and organic mercurials. N. Y. Agr. Exp. Stat. Bull. 183. 1931.
2. ———. Vegetable seed treatment. N. Y. Agr. Exp. Stat. Bull. 597. 1931.
3. HORSFALL, JAMES G., A. G. NEWHALL, and C. E. F. GUTERMAN. Dusting miscellaneous seeds with red copper oxide to combat damping-off. N.Y. Agr. Exp. Stat. Bull. 643. 1934.
4. ———. Dusting tomato seed with copper sulphate monohydrate for combating damping-off. N. Y. Agr. Exp. Stat. Bull. 198. 1932.
5. ———. Red oxide of copper as a dust fungicide for combating damping-off by seed treatment. N. Y. Agr. Exp. Stat. Bull. 615. 1932.
6. MASSIE, I. On the presence of hibernating mycelium of *Macrosporium solani* in tomato seed. Royal Bot. Gard. Kew. Bull. Misc. Inf. 4: 1–3. 1914.
7. MILLER, J. H. Tomato disease notes for Georgia for 1936. Plant Dis. Rptr. 20: (22). 1936.
8. ———, and WILLARD F. CROSIER. Pathogenic associates of tomato seed: their prevalence, relation to field disease, and elimination. Proc. Assoc. Off. Seed Anal. N. A. 1936.

9. MOORE, M. B., *et al.* Cooperative vegetable seed treatment tests in 1940. Committee for coordination in cereal and vegetable treatment research of The American Phytopathological Society. Extension Path. 44: 1–13. 1940.
10. MOORE, W. D. Some factors affecting the infection of tomato seedlings by *Alternaria solani*. Phytopath. 32: 399–403. 1941.
11. SAMSON, R. W. Disease-free tomato seed. Report of the Director. Ind. Agr. Exp. Stat. 1937.
12. ————. Seed transmission of early blight of tomatoes. Report of the Director. Ind. Agr. Exp. Stat. 1938.
13. ————, and H. REX THOMAS. Tomato diseases in Indiana. Ind. Agr. Exp. Stat. Cirs. N. 257. 1940.
14. ————, T. J. NUGENT, and L. C. SHENBERGER. Importance of seed transmission of early blight and Fusarium wilt of tomatoes. Phytopath. 32: 16, No. 1. 1942.
15. VAN HALTERN, FRANK. Control of tomato seedbed diseases of southern plants. Ga. Agr. Exp. Stat. Bull. 187. 1935.
16. ————. Crop rotation for tomato plants. Ga. Exp. Stat. Press Bull. 476. 1939.

# OBSERVATIONS ON THE BACTERIAL CROWN, STEM, AND BUD ROT OF DELPHINIUM

STEPHEN WILHELM[1]

(Accepted for publication January 31, 1943)

The stem and bud rot caused by *Erwinia phytophthora* (Appel) Comm. S. A. B. has been destructive for several years in commercial and garden plantings of rocket larkspur (*Delphinium ajacis* L.) in California (1). Also, it frequently has caused serious losses in plantings of perennial delphinimum, producing a crown rot of the type illustrated by Blodgett (2). On either of these hosts the disease may under favorable environmental conditions assume epidemic proportions. Opportunity has been afforded during the last 3 years for observations of conditions favoring this disease. Because production of larkspur seed has been increased to supply medicinal needs of the armed forces, these observations are presented at this time.

The symptoms of this disease have been adequately described for perennial delphinium (2, 7) and larkspur (1, 4) and are not, therefore, discussed in this paper.

Excessively wet soil greatly increases the injury to infected plants and facilitates spread of the bacteria, as observations in June, 1942, in Santa Barbara County clearly showed. In one seed field of rocket larkspur, in which the lower third of the planting had been watered unintentionally by irrigation waste from an adjoining field at the time the flower buds were forming, 20 per cent of the plants in the wet area were infected (sample of 400 counted). No infected plants were found in the rest of the planting in which the soil was dry and hard. In another such field, irrigated a few days before, the lower ends of the rows had been flooded and were much wetter than the rest of the field. In the flooded area 59.5 per cent of the plants were infected (sample of 242 counted) and in the irrigated, but relatively drier portion, 7.4 per cent were infected (sample of 446 counted). In a third field of larkspur a low area, adjacent to an irrigation standpipe, had no plants in the center, nearly all plants diseased at the margin, and only an occasional diseased plant in the surrounding field (Fig. 1). These observataions agree with those on blackleg of potato caused by the same organism (6).

Similarly, the incidence of bacterial crown rot in perennial-delphinium plantings is greatly increased by excessively wet conditions. An experimental planting that had been watered by overhead sprinklers, when observed in West Los Angeles in June, 1940, was so severely injured that several types were totally destroyed. As soon as such watering was discontinued the losses sharply declined. In the same year a nearby commercial planting of perennial delphinium suffered similar losses, being heavily irri-

[1] The writer is grateful to K. F. Baker for direction, advice, and kind criticism received in the preparation of the manuscript.

gated in furrows at rather frequent intervals. Only mild losses have been sustained in both the experimental and commercial plantings in the 2 years since, probably because the irrigation has been kept to an absolute minimum, particularly after elongation of the flower spike begins. Observations during the same period in other nearby plantings where irrigation was not regulated and where serious losses from this disease occurred, indicated that these seasons were favorable to the disease. In Southern California it is usually necessary to irrigate perennial delphinium in order to obtain maximum growth. It is possible to irrigate with only negligible losses by making the furrows some distance from the rows and having the crowns of the plants on low ridges.

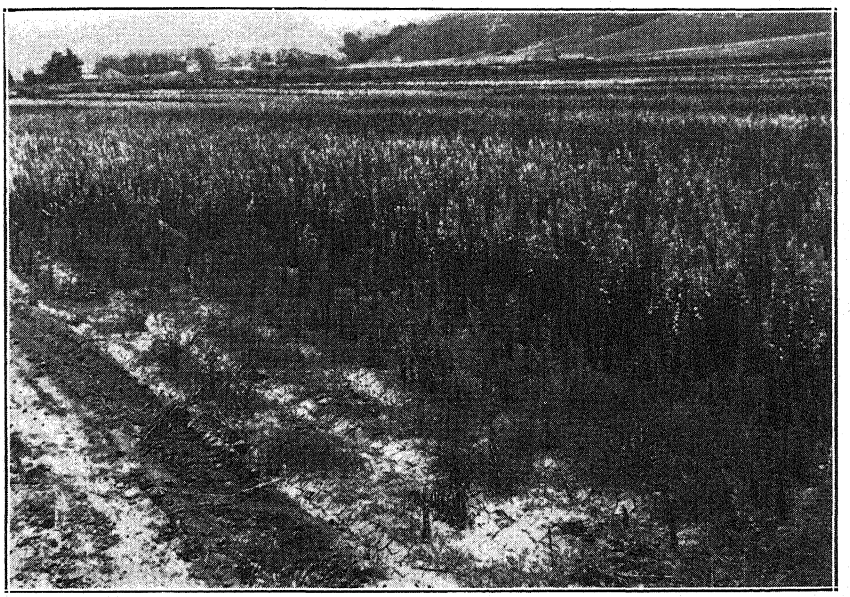


FIG. 1. Part of a field planting of rocket larkspur that had been flooded from a nearby irrigation standpipe, showing the increased severity of the disease resulting from excess water. Photographed June 16, 1942.

The cracking of the stems of perennial delphinium at or near the soil level appears particularly to predispose them to infection. Such cracks are illustrated by Blodgett (2). Since these cracks appear at the time the flower spike begins to elongate, this developmental stage may be regarded as the critical period for this disease. This is also true for rocket larkspur (1). Observations indicate that fluctuating conditions of soil moisture and perhaps temperature aggravate this apparently natural cracking. Under wet, poorly aerated conditions such cracks do not heal, and may serve as open avenues of infection. These observations are in general agreement with those on the healing of cut seed-pieces in relation to potato blackleg (6). Leonian (7) states that young delphiniums may be planted successfully in the spots where old, diseased plants died. Since stem cracking occurs in late

stages of growth, the escape of young plants is perhaps to be expected. However, such replanting would hardly seem an advisable garden practice.

The disease has been consistently most damaging in the summer months, and is regarded by investigators (2, 5, 7) and growers as favored by warm weather. While this seems to be a correct generalization, it should be recorded that losses can be severe during winter months. In February, 1941, after several months of heavy rain, practically all of the abundant volunteer larkspur plants in a field in Santa Barbara County were found infected and many killed.

Observations show that soil carry-over may greatly increase the incidence of the disease. In 1942, a strip 45 yards wide along one end of a field of

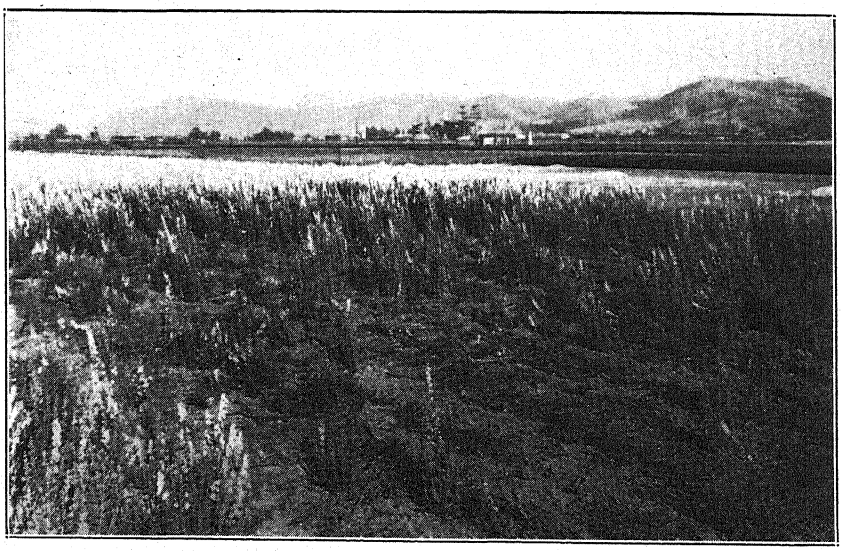


FIG. 2. Field planting of rocket larkspur variety Super Majestic Rose, showing in the foreground the area which had been planted to larkspur the previous season and in the background the area not previously planted to this crop. Photographed June 16, 1942.

rocket larkspur extended into an area in which the same plant had been grown the previous year. The plants occurring in this strip showed a high incidence of disease, the line of demarcation separating the diseased plants from the healthy coinciding closely with the margin of the overlap. In the overlapped planting, Larkspur var. Blue Spire showed 58.0 per cent, diseased plants, Lustrous Carmine in one block 54.0 per cent and in another 92.8 per cent, Los Angeles 71.7 per cent, and Super Majestic Rose 93.6 per cent (Fig. 2). In all varieties the plants counted as healthy were badly wind beaten or broken over, so that from the standpoint of seed production the area was practically a total loss. In the clean planting there was little or no evidence of the disease. The causal organism apparently is able to live over in soil with a high degree of infectivity from one planting cycle to the next, a period of at least 6 months. From the standpoint of field control,

therefore, it would be a wise precaution to practice a crop rotation of at least 1 year and preferably 2. However, it should be pointed out that in some cases the disease incidence may be high in plantings where delphiniums have never previously been grown. In the experimental planting of perennial delphiniums mentioned above, the young plants were grown in pasteurized soil and transplanted into a field which had not been cultivated for at least 10 years and which apparently had never before been planted to delphiniums. All plants of a number of varieties were killed. Similar experiences are reported by Blodgett (2).

The hot-water treatment of rocket larkspur seed that has been recommended (1) for this disease might reasonably be effective through reduction of inoculum potential, but evidence on this point in commercial seed fields is somewhat conflicting. It is expected that it would be more effective on soils of low than of high inoculum potential.

The observations reported above indicate that two variable factors, soil moisture and inoculum potential, may determine the incidence of this disease in the field during the summer months. Under conditions of low inoculum potential no disease or only slight losses occur under conditions of low soil moisture but severe losses may occur, though tardily, if the soil be waterlogged; this situation is illustrated in figure 1. Under conditions of high inoculum potential, prompt, heavy losses result if the soil receives more than a minimum of water, but the same amount of water may be applied safely to soil of low inoculum potential; this condition is illustrated in figure 2. Soils of heavy inoculum potential have been successfully utilized for perennial delphinium by applying a minimum of water at a distance from the crowns. It is also in the interest of maintaining a low inoculum potential in the soil to avoid overirrigation.

Because commercial seed plantings in California are made before or during the rainy season it is not always possible to control the factor of soil moisture, and crop rotation must be practiced. In California home gardens it is customary to plant seedlings of rocket larkspur rather than seed and planting may, therefore, be delayed until the moisture factor may be effectively controlled. Rotation, while desirable, may not in such cases be imperative. Seedlings of perennial delphinium transplanted to infested soil in early spring may be successfully grown through the first season by careful attention to watering; they are generally not grown for a second season in California because of the high percentage of aster yellows that appears. Application of Bordeaux mixture (4, 9) and mercuric chloride (9) around the crowns of delphiniums has been suggested for control of this disease but has not been tested in California.

With rocket larkspur the black stem streaking, characteristic of diseased plants, often does not extend down to soil level, suggesting transmission of the disease through some aerial agency. Seed transmission and subsequent splashing of bacterial exudate from plant to plant during foggy or wet windy weather are possible explanations, but insect vectors may be involved

(1). Slugs have also been suggested (9) as carriers of the bacteria. Trogoderma beetles were found associated with the bacterial exudate from stems of perennial delphinium in Idaho (2). Greenhouse tests have shown that when mature parts of rocket larkspur plants are sprayed with a bacterial suspension, they do not develop symptoms, infections occurring only in the growing apices and developing buds after an incubation period of about 1 week. These results agree with the observations of Chester (3) on the nonoccurrence of stomatal infection. Plants hypodermically inoculated at the base, however, may be totally destroyed within 30 hours.

Delphiniums have been noted to differ considerably in susceptibility to the bacterial stem and bud rot. Varieties of rocket larkspur that have been particularly susceptible in field plantings are: The Dazzler, Miss California, Lilac King, Stock Flowered Dark Blue, Blue Bell, Super Majestic Rose, Lustrous Carmine, and Los Angeles. Of the perennial delphiniums,[2] the Belladonna group, represented by the varieties Belladonna, Bellamosum, and Lamartinii, is highly susceptible under field conditions, the infection often occurring high on the stem. Of the *Delphinium elatum* L. group, the large candelabrum types listed as Pacific Giant Hybrids, the English (Wrexham) hollyhock strain, and the Blackmore and Langdon strains, are all susceptible. The Grandiflorum group, including the varieties listed as *D. chinensis* Hort. and the spurless types listed in the trade as *D. cinereum* Hort. and *D. tatsienense* Hort., all appear under field conditions to be highly resistant. Of California native delphiniums, *D. cardinale* Hook., *D. hesperium* Gray, *D. parryi* Gray, and *D. scopulorum* var. *glaucum* Gray, have been noted as being very susceptible under garden conditions. Varietal differences in susceptibility of perennial delphinium were also noted by Blodgett (2) but details were not reported.

## SUMMARY

The bacterial crown rot of perennial delphinium and the stem and bud rot of rocket larkspur, caused by *Erwinia phytophthora*, are strongly favored by excessive soil moisture. It is possible to greatly reduce losses from these diseases by application of a minimum of water by means of furrows at a distance from the rows, and by planting on low ridges. Particular care in irrigation is necessary after the flower spikes begin to elongate.

The bacteria are able to live over in soil in a high degree of infectivity at least from one planting cycle to the next and crop rotation is, therefore, advisable in the commercial production of seed and, where possible, in ornamental plantings. The disease may become destructive on soils of low inoculum potential if the soil is saturated with water; in the interest of maintaining a state of low infectivity in soil excessive irrigation should be avoided.

[2] Information on the perennial types was kindly supplied by G. A. L. Mehlquist. The taxonomic arrangement followed is that of Mehlquist, Blodgett, and Bruscia (8).

While the disease definitely is worse on both hosts in the summer months it may become destructive during the winter.

The time of flower-spike elongation is the critical period for this disease in the life of both types of delphinium. This seems to be correlated with the appearance of deep cracks in the stem bases.

Varietal differences in susceptibility to this disease have been noted in both rocket larkspur and perennial delphinium.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
LOS ANGELES, CALIFORNIA.

LITERATURE CITED

1. ARK, P. A., C. M. TOMPKINS, and R. E. SMITH. A bacterial bud and stem rot of rocket larkspur. Phytopath. 28: 281–283. 1938.
2. BLODGETT, E. C. A disease of delphinuium. Amer. Delphinium Soc. Book 1939: 88–91. 1939.
3. CHESTER, F. D. A bacterial disease of delphinium. Phytopath. 27: 855–858. 1937.
4. DODGE, B. O. A bacterial disease of *Delphinium ajacis*. Jour. N. Y. Bot. Gard. 36: 257–260. 1935.
5. LASKARIS, T. Report of the delphinium crown rot investigation fellowship. Amer. Delphinium Soc. Book 1939: 102–108. 1939.
6. LEACH, J. G. Potato blackleg: the survival of the pathogen in the soil and some factors influencing infection. Phytopath. 20: 215–228. 1930.
7. LEONIAN, L. H. Diseases of delphinium. Bacterial crown rot. *In* The Garden of Larkspurs, by L. H. Bailey. pp. 28–29. Macmillan Co. (New York). 1939.
8. MEHLQUIST, G. A. L., C. O. BLODGETT, and L. BRUSCIA. Colchicine-induced tetraploidy in *Delphinium cardinale* Hook. Jour. Hered. (in press).
9. MULFORD, F. L., and F. WEISS. Culture and diseases of delphinium. U. S. Dept. Agr. Farmers' Bull. 1827. 1939.

# ARMILLARIA ROOT ROT OF FRUIT TREES IN THE EASTERN UNITED STATES

J. S. Cooley

(Accepted for publication March 5, 1943)

When the writer began work on the problem of root rot of deciduous fruit trees in 1931, Armillaria root rot was included in the project. The world-wide distribution of this disease and its importance in the western part of this country made it seem desirable to ascertain to what extent it is responsible for the loss of peach trees in the eastern part of this country. Armillaria was known to be present in the Sand-hill region of North Carolina and adjacent States, where heavy losses from root diseases of peaches occurred.

This paper reports a study of Armillaria root rot, giving the results of field surveys on distribution and severity, also results of inoculation experiments with peaches, apples, and pears, carried out in Virginia, Maryland, and replanting experiments with peaches in North Carolina.

## REVIEW OF LITERATURE

Very early in the history of plant pathology, Armillaria was recognized by Hartig (4) in Germany as causing a root disease. Since that time it has been reported from many regions widely separated over the world, and many papers have appeared dealing with the disease. Wallace (8) states that close to 200 host plants for this pathogen have been recorded in the literature.

The extent of the literature on this disease is shown by Reitsma (6) who lists 494 citations, and more have appeared since the publication of his paper in 1932.

Various workers on this disease have reported widely divergent views on the virulence of its pathogenicity. Many workers have considered Armillaria an active parasite. Hendrickson (5) states, with reference to prunes, that the vigor of an individual tree is without effect on resistance to the disease.

Some of the workers, particularly the more recent ones, hold a different view concerning the parasitism of Armillaria. Reitsma (6) states: "It is a facultative parasite with properties of a perthophyte."

Day (3) states that Armillaria is a secondary parasite on the oak, spruce, and fir, with such predisposing factors as mildew on the oak and drought and sun-scorch on the fir and spruce. He reports a Corsican pine affected by the fungus but showing no evidence of serious injury so long as it occupied a dominant forest position. After losing its dominance by growth of younger trees, this tree was rapidly invaded and killed by Armillaria.

Butler (1) reports that collar crack of tea bush in Africa, caused by Armillaria, results in serious loss only after the soil becomes infertile from

erosion. Dade (2) reports that this disease on the cacao in Africa is epidemic only on soil of excessively high moisture content.

This diversity of opinion concerning the pathogenicity of this fungus may be due to an extreme variation in strains of the organism or it may be due to differences in environmental conditions favoring or retarding the resistance of the host. Although it is not possible to separate the host and parasite in their relationships to environment, the observations and experiments reported in this paper indicate that unfavorable environment for the peach as host is especially important in inducing susceptibility.

## SYMPTOMS OF ARMILLARIA ON PEACH AND OTHER STONE-FRUIT TREES

The leaves of trees affected by the Armillaria disease roll along the midrib, and have a wilted appearance. Because the internodes are much shortened and growth is greatly retarded, the trees have a stunted appearance. In the later stages the limbs on the same side as the affected roots almost cease growth, are a light green, or may die.

This disease, like other root disturbances, cannot be positively identified by the visible symptoms on the above-ground parts, for the symptoms on the foliage may be similar to those produced by other root troubles, such as root winter injury and collar winter injury, or root suffocation.

The most characteristic symptom on stone-fruit trees is the white to cream-colored mycelial growth in the cambium of the affected roots or root crown. On margins of the diseased area the fungus may spread out in the cambium in distinctive fan-shape pattern. The fungus also advances into the wood of affected roots, producing a decay that becomes progressively lighter, eventually turning to a very light tan or cream color.

Another symptom of the disease is the presence of black rhizomorphs adhering to the surface of affected roots. These rhizomorphs advance through the soil and thus spread the disease from root to root and tree to tree. Of course, one of the most definite diagnostic characters of the disease is the fruiting body of the fungus, which sometimes forms about the collar of affected trees or on shallow roots. In culture, the fungus is readily distinguished from most other fungi by the formation of honey-colored rhizomorph-like structures permeating the agar.

## ORCHARD SURVEYS ON THE PREVALENCE OF THE DISEASE ON POME AND STONE-FRUIT TREES

From 1931 to 1942, in connection with other root-rot studies, surveys on the incidence of this root rot have been made in Virginia, Maryland, North Carolina, and other eastern and central States. Since Armillaria occurs as a saprophyte in the deciduous forests in these regions and is known to rot the roots of a wide variety of cultivated plants, including the apple and the peach, an inspection to determine its presence and pathogenicity on apple and peach trees was made at every opportunity. The results of these obser-

vations were different from what might have been expected, judging from the literature on the general distribution of this disease.

### Armillaria on Apple Trees

In the Hood River Valley, Oregon, Armillaria has been observed by the writer, growing on the collar of apple trees affected with the sunscald type of winter injury on the trunk, but casual observation indicated that it was not active as a parasite on the roots of uninjured apple trees. In the eastern part of the United States surveys for various root disturbances have been made in many of the fruit districts. In only one case was Armillaria found growing on apple roots. This tree, which was located in Virginia, was growing under adverse conditions because of wet, poorly drained soil.

### Armillaria on Cherry and Peach Trees

Surveys have been made of the cherry orchards in northwestern Pennsylvania, but no Armillaria root rot was observed. In one case a cherry tree that had been planted on newly-cleared land in Maryland was killed by Armillaria root rot. The observations on cherry trees, though limited, indicate that Armillaria root rot is not of frequent occurrence or serious in the eastern part of this country.

Observations on root diseases of the peach have been made in a number of peach orchards in Georgia and in the Shenandoah-Cumberland region of Pennsylvania, Maryland, and Virginia, but Armillaria root rot was not found.

In the Coastal Plain of Maryland, Armillaria root rot was occasionally found. Annual observations on an affected tree have been made during the past 8 years and are here recorded. In 1933, a 3-year-old peach tree replant in a 6-year-old peach orchard, growing in sandy soil in the Coastal Plain of Maryland was found to be diseased with Armillaria and was removed. The next year an adjacent tree, also a replant, showed disease symptoms and an examination, together with isolations, showed the presence of Armillaria, several roots being affected at that time. This tree was allowed to stand and examinations have been made each year since then to find out how rapidly the disease spread to sound roots and the time required to kill the tree. Although the disease has been gradually advancing, the tree has been able to make new roots above the diseased ones and was still alive in 1942. In 1941, the tree ceased active growth but the poor vigor was at least partly due to borers. In spite of the massive invasion and, presumably, an abundance of inoculum in the soil, the spread of the disease to other roots has not been rapid. The fact that a diseased tree remained alive at least 8 years after it was known to be infected and that the adjacent trees have not given indication of being affected, indicates that the fungus has not invaded rapidly and that conditions have not been favorable for spread to or infection of adjacent trees. These two trees were growing in a slight depression where there was some evidence of slow drainage after heavy precipitation.

In the sand-hill section of North Carolina, Armillaria was frequently found on peach trees. In this region most of the peach orchards were set on land newly cleared of partly deciduous forest, thus providing favorable conditions for an abundant growth of the pathogen in the soil. Certain adverse host conditions, however, existed. The soil is very light and sandy and is probably low in humus and mineral plant foods. Also, climatic conditions have tended toward temperature and moisture extremes. An injury about the collar, seemingly winter injury, was prevalent. Furthermore, nematode injury contributed to the complexity of the situation. It was difficult therefore to determine to what extent Armillaria was the primary cause of the death of affected trees.

## INOCULATION EXPERIMENTS

Inoculation work was begun in 1933 and has been carried on for 9 years, using 1- to 5-year-old nursery trees of apple, pear and peach growing at Arlington Experiment Farm, Arlington, Virginia, at the Bureau of Plant Industry Station, Beltsville, Md., and at Fruitland Orchard, Hamlet, North Carolina. The inoculum used at first was a pure culture of the fungus, obtained from apricot root from The Dalles, Oregon, and grown on peach twigs about 2 inches long and $1\frac{1}{2}$ inches in diameter. It was expected that the use of such large pieces would favor production of rhizomorphs, which Thomas (7) states are necessary for infection. In the later inoculation work the inoculum was grown on pieces of apple limb an inch or more in diameter and about 2 inches long, that had been placed in jars with wheat bran, then sterilized and subsequently inoculated. After several months, when the wood was well covered with the fungus, inoculation was made by scraping the outer bark of the root with a trowel, laying a piece of the inoculum on the root, and replacing the soil. This method of inoculation was suggested by Harold Thomas and Donald Bliss of the University of California.

In 1934, at Arlington Experiment Farm, periodic monthly inoculations were made on apple, pear, cherry, plum, and peach trees that had been growing in the nursery for 3 years. Many of the peach trees used in later inoculation work were of bearing age, but were growing in close nursery formation.

In 1938, an experimental plot of peach trees was set at Hamlet, N. C., with 1-year-old peach trees of a known root and top combination, the roots being seedlings of known commercial varieties. These trees were set 4 feet apart in rows 8 feet wide in a plot in a 10-year-old peach orchard where the trees had died. Armillaria was present on some of the old peach trees still standing only a short distance from the experimental plot. The cause of the death of old trees in that part of the orchard was not definitely determined. The conditions obtaining there were probably typical of many other orchards where dead trees occurred. The next year after the trees were planted the roots were inoculated in July with a strain of the fungus from North Caro-

lina. The next year inoculation was made on the opposite side of the main root.

The results of all these inoculations in North Carolina, Virginia, and Maryland, were, in the main, negative. In a few cases, lesions as much as 10 mm. in diameter were produced at the point of inoculation on cherry or peach-tree roots; however, these lesions soon healed over. The results of all apple and pear inoculatons were negative. At the Hamlet plot, some trees died the first year because of late planting and hot weather. Some trees died each year but there was no evidence that any root-rot pathogen killed the trees.

## DISCUSSION OF RESULTS

The fact that the isolates used for inoculum were taken from a variety of sources, including widely separated geographical regions, suggests that the absence of infection was due to host resistance rather than failure to obtain normally virulent strains of inoculum. These negative inoculation results agree with field observations in eastern United States in indicating that the disease occurs rarely and that the edaphic conditions where most of these observations were made were not suitable for host infection. Where it does occur there is evidence that the host has been weakened by an unfavorable environment.

The references in the literature to Armillaria as causing a serious root disease of cultivated plants are much more likely to emanate from a region of low precipitation, as South Africa or West Africa or California, than from a more humid region.

The evidence given above indicates that the comparative absence of the disease in the eastern United States and its prevalence in the West Coast regions are due to host reaction to physical environment. That is, the environmental conditions in the West Coast regions are favorable to host susceptibility, and in the eastern regions conditions are, in the main, unfavorable to susceptibility. It may be that the xeric conditions of the West favor host susceptibility to this pathogen.

## SUMMARY

Surveys in many of the fruit regions in the eastern and central parts of the United States resulted in occasionally locating stone-fruit trees attacked by Armillaria. The pathogen has been isolated from peach roots from the Sand-hill region of North Carolina, but no evidence has been obtained showing whether Armillaria is the initial cause of the decline of the trees or whether the fungus follows some disorder caused by adverse environment.

Observations for 8 consecutive years on a diseased peach tree growing in the Coastal Plain of Maryland showed a slow advance of Armillaria in the affected tree, but no indication of spread to the surrounding trees.

At monthly intervals for 2 years inoculations were made on young pome and stone-fruit trees. These usually gave negative results, but in some in-

stances small lesions were formed. These healed by the next year. Inoculation of replants in an old peach orchard in North Carolina in which Armillaria was present also failed to produce the disease. Natural infection has not occurred on these test trees.

PLANT INDUSTRY STATION,
BELTSVILLE, MD.

LITERATURE CITED

1. BUTLER, E. J. Report on some diseases of tea and tobacco in Nyasaland. Dept. Agr. Nyasaland. 1928.
2. DADE, H. A. Collar crack of cacao (*Armillaria mellea* Vahl. Fr.). Dept. of Agr. Gold Coast Bull. 5. 1927.
3. DAY, W. R. Environment and disease. A discussion on the parasitism of *Armillaria mellea* Vahl. Forestry 3: 94–103. 1929.
4. HARTIG, ROBERT. Wichtige Krankheiten der Waldbaume, pp. 125–126. (Berlin.) 1874.
5. HENDRICKSON, A. H. Oak fungus in orchard trees. Calif. Agr. Exp. Stat. Circ. 289: 1–13. 1925.
6. REITSMA, J. Studien über *Armillaria mellea*. Phytopath. Zeitschr. 5: 461–522. 1932.
7. THOMAS, H. E. Studies on the nature of host resistance to *Armillaria mellea*. Phytopath. 19: 1140. 1929.
8. WALLACE, G. B. Armillaria root rot in East Africa. East Afr. Agr. Jour. 1: p. 182. 1935.

# INFLUENCE OF CONTACT PERIOD ON THE PASSAGE OF VIRUSES FROM CION TO STOCK IN TURKISH TOBACCO

C. W. BENNETT

(Accepted for publication November 30, 1942)

## INTRODUCTION

Extensive evidence indicates that there are distinct differences between viruses in their relationships to the tissues of the plants in which they occur. Those viruses that cause generalized yellowing, leaf curling, leaf rolling, etc., without mottling, appear as a rule to be more or less limited to the phloem of the invaded plants and the symptoms produced are typical of disturbances arising in the phloem. Those viruses that cause mottling and various types of local lesions occur in relatively high concentrations in the parenchyma and produce symptoms typical of disturbances arising in the parenchyma. However, it is evident that most viruses of this latter type occur also to some extent in the phloem, although their concentration in this tissue may be relatively low as compared to that in the parenchyma.

It would be expected that viruses of these two groups would exhibit differences in their ability to pass from an infected cion or bud to a healthy stock through a graft union. Obviously, if a virus is limited to the phloem it cannot pass from a diseased cion to a healthy stock until a phloem bridge is formed across the graft union; whereas a virus that increases and moves in parenchyma should be able to pass the graft union as soon as a parenchymatous bridge becomes available, perhaps considerably before there is differentiation of phloem in the tissue uniting stock and cion.

Evidence that may be interpreted as support for this hypothesis was obtained by Kunkel[1] who showed that a shorter contact period was required for the transmission of peach mosaic than for that of peach yellows, little-peach, and peach rosette. No such studies, however, are available on herbaceous plants, possibly because of the difficulty of selecting viruses with the desired tissue relationships and at the same time not transmissible by manipulation necessary in making grafts. Recently, two viruses have been found, one a strain of a ring-spot virus and the other a strain of cucumber-mosaic virus that are transmissible to Turkish tobacco by juice inoculation, only with considerable difficulty. These two viruses have been compared with the virus of curly top with respect to the readiness with which they will pass from an infected cion to a healthy stock. The results of these experiments are presented in this paper.

## MATERIALS AND METHODS

The strain of curly-top virus used in these experiments was one that is relatively severe on sugar beet and causes severe symptoms on Turkish

[1] Kunkel, L. O. Contact periods in graft transmission of peach viruses. Phytopath. 28: 491–497. 1938.

tobacco. The virus has been carried in sugar beet and tobacco for several years.

The ring-spot virus came originally from field tomatoes but has been held in Turkish tobacco for more than two years. Repeated attempts to transmit this virus to Turkish tobacco and tomato by juice inoculation have resulted in only a few cases of infection. In a few instances limited numbers of local lesions were produced on Turkish tobacco but in most cases neither local lesions nor systemic infection resulted from juice inoculation. Systemic infection did not always follow the production of local lesions. Plants that are systemically infected produce rings and spots of necrotic tissue on the younger leaves as primary symptoms after which the plant recovers to the extent that no obvious symptoms are evident on the subsequent growth.

The strain of cucumber-mosaic virus used came from sugar beet on which it causes a severe form of mottling. It is transmissible to Turkish tobacco by juice inoculation but only very small percentages of the inoculated plants have become infected.

Four lots of diseased Turkish tobacco plants were prepared as sources of diseased cions used to make the graft inoculations. Lot 1 was infected by curly-top virus; lot 2 by ring-spot virus; lot 3 by curly-top virus plus ring-spot virus; and lot 4 by curly-top virus plus ring-spot virus plus cucumber-mosaic virus. Healthy Turkish tobacco plants were grown to a height of about 2 feet. The stems were cut back to a height of about 10 inches. Cions from the 4 lots of diseased plants were attached at the tops of the stems, the area of contact between cion and stock being a slanting surface somewhat more than an inch in length. Cions were removed after the alloted period of contact. The contact periods between cion and stock varied from 1 to 14 days with a 1-day interval for the first 6 days and a 2-day interval thereafter.

## EXPERIMENTAL RESULTS

The detail results of this series of tests are shown in table 1. It will be noted that no curly-top infection occurred in plants in which the union with diseased cions was less than 5 days. In general there was an increase in percentage infection as the time of contact with diseased cions was increased. Infections by the viruses of ring spot and cucumber mosaic began with the plants having a 2-day contact period with infected cions, and increased rapidly as the period of contact was increased. The fact that no infection took place on any of the plants in which the contact period was 1 day indicates that there was no infection resulting from handling the plants during the process of making the grafts.

Infection by curly-top virus and ring-spot virus from cions that carried both viruses closely parallels that obtained where the two viruses were in separate cions. This is true also from the cions that had all 3 viruses. There is some evidence, however, from the results obtained from the cions containing all 3 viruses that the ring-spot virus moves more rapidly

into the healthy stock than does the cucumber-mosaic virus. This is evident from the figures showing infection. Analysis of individual plant records showed that 23 of the plants inoculated with the triple virus combination were infected by only one virus. Of these, 19 were infected by ring-spot virus and only 4 by cucumber-mosaic virus. Further tests in which the viruses of ring spot and cucumber mosaic were combined in the same cion have given similar results. Of 123 plants infected by only one virus, 102 were infected by ring spot and 21 by cucumber mosaic. There was no instance of infection by curly top alone where this virus was in combination with one or both of the other viruses.

TABLE 1.—*Influence of period of contact on passage of viruses from diseased cion to healthy stock in Turkish tobacco*

| Period of contact between cion and healthy stock | Plants infected[a] from cions carrying the indicated viruses and combination of viruses | | | | | | |
|---|---|---|---|---|---|---|---|
| | Curly-top virus | Ring-spot virus | Curly-top virus plus ring-spot virus | | Curley-top virus plus ring-spot virus plus cucumber-mosaic virus | | |
| | Curly top | Ring spot | Curly top | Ring spot | Curly top | Ring spot | Cucumber mosaic |
| *Days* | *Number* | *Number* | *Number* | *Number* | *Number* | *Number* | *Number* |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 3 | 0 | 6 | 0 | 2 | 2 |
| 3 | 0 | 9 | 0 | 8 | 0 | 10 | 6 |
| 4 | 0 | 13 | 0 | 11 | 0 | 15 | 10 |
| 5 | 2 | 15 | 1 | 12 | 0 | 15 | 10 |
| 6 | 5 | 15 | 4 | 13 | 2 | 15 | 15 |
| 8 | 7 | 15 | 6 | 15 | 6 | 15 | 14 |
| 10 | 9 | 15 | 10 | 15 | 7 | 15 | 15 |
| 12 | 11 | 15 | 12 | 15 | 11 | 15 | 15 |
| 14 | 14 | 15 | 15 | 15 | 14 | 15 | 15 |

[a] Figures represent the number of plants infected out of 15 inoculated in each instance.

## DISCUSSION AND CONCLUSIONS

The results of this series of graft inoculations shows clearly that the virus of ring spot and the virus of cucumber mosaic can be separated readily from the virus of curly top by taking advantage of the contact period required for passage of the respective viruses through a graft union. Also the virus of ring spot often is separable from the virus of cucumber mosaic in short contact periods. Less often the virus of cucumber mosaic is separated from that of ring spot. It is believed that these separations are due, for the most part, to the difference in tissues in which the viruses are able to travel.

Previous histological investigations of the graft unions of Turkish tobacco have shown that there is at least a meristematic union between stock and cion after 48 hours of contact and that phloem begins to differentiate in the new tissue of the union at least by the seventh day.

Strong circumstantial evidence indicates that parenchyma-inhabiting viruses are able to move from cell to cell in the parenchyma through the plasmodesmata. This would permit the viruses of cucumber mosaic and ring spot to move across the graft union as soon as a parenchyma union became available. The curly-top virus, however, appears to be more or less limited to the phloem, and it is known that it is unable to move through at least certain types of parenchyma. It seems probable, therefore, that the virus of curly top is retained in the cion until differentiation of phloem begins in the graft union.

The apparent greater rate of movement of the ring-spot virus through the graft union as compared with that of the cucumber-mosaic virus might be due to greater mobility resulting from size of particle, rate of increase, or other factors. However, there is a basis for suggesting that this also may be the result of the relation of the respective viruses to tissues. Valleau[2] is of the opinion that the meristematic tissue of tobacco plants is invaded by the ring-spot virus. Apparent occurrence of this virus in pollen and the fact that infected plants recover from severe initial symptoms of the disease support this concept.

Sheffield's[3] work indicates that the virus of aucuba mosaic of tomato does not completely invade the meristematic cells of the growing point of affected plants but progressively invades the cells below the primary meristem as they begin to differentiate into more mature tissues. Some cells are invaded early enough to inhibit development of plastid primordia, others are not. This unequal invasion in which development of plastid primordia is suppressed in early invaded cells and not in later invaded cells, results in the mottled condition of the leaves of affected plants.

If the ring-spot virus is able to invade apical meristem, as Valleau suggests, and if mottling in general results from delayed non-uniform invasion of the cells below the primary meristem of the growing apex of the stems, as Sheffield's results would tend to indicate, it is probable that the ring-spot virus may be able to invade and migrate through cells that are somewhat younger than those that will permit passage of the virus of cucumber mosaic. Hence it is quite possible that the virus of ring spot might pass through graft unions when a meristematic union is available; whereas passage of the virus of cucumber mosaic would be delayed until meristem begins to differentiate into other tissues. In plants grafted to cions carrying both cucumber-mosaic virus and ring-spot virus this might account for the larger number of plants in which only ring-spot virus occurred.

It is true, of course, that some of the plants grafted to cions containing both viruses showed only cucumber mosaic; and it may be argued that in these instances the virus of cucumber mosaic passed the graft union first.

[2] Valleau, W. D. Experimental production of symptoms of so-called recovered ring-spot tobacco plants and its bearing on acquired immunity. Phytopath. 31: 522–533. 1941.

[3] Sheffield, F. M. L. The development of assimilatory tissue in Solonaceous host plants affected with the aucuba mosaic of tomato. Ann. Appl. Biol. 20: 57–69. 1933.

This possibility is by no means eliminated, but it is not necessarily true. As was noted earlier, there is evidence indicating that local infection by the strain of ring-spot virus used does not always result in systemic infection. Therefore, even with plants showing only cucumber mosaic, the virus of ring spot may have passed through the graft union first, but failed to establish itself sufficiently to cause systemic infection.

On the basis of information already available on graft transmission of "yellow" and "mosaic" types of viruses it seems reasonable to expect that the mosaic types in general will be found to be transmissible by short contact periods between diseased and healthy tissue. Apparently a parenchymatous union, and perhaps sometimes only a meristematic union, is sufficient to permit virus passage. Such unions might occur between tissues of widely different species of plants. Even though the grafted tissue failed to remain alive for more than a few days it might make a union that would permit passage of virus. If this should prove to be true the graft technique, if extensively tested, might prove to be of considerable value in extending knowledge as to virus host range and in determining virus relationships, in groups of plants such as Rosaceae, with viruses that are not juice transmissible and for which insect vectors are unknown or have limited host ranges.

U. S. SUGAR PLANT FIELD LABORATORY,
RIVERSIDE, CALIFORNIA.

# CARNATION MOSAIC

D. B. CREAGER[1]

(Accepted for publication January 2, 1943)

A disease of carnations (*Dianthus caryophyllus* L.) with symptoms resembling those of mosaic diseases of other plants, first came to the attention of the writer in the fall of 1939. Since then it has been observed frequently, affecting many of the leading commercial varieties of greenhouse carnations.

Others previously had encountered maladies of carnations quite similar to, if not identical with, the disease referred to here as carnation mosaic. As early as 1912 Peltier (5), working at the University of Illinois, became interested in a disease that he referred to as carnation yellows. He described some leaf symptoms that appear to be very similar to those associated with carnation mosaic, but he did not mention flower breaking. In a limited test, Peltier grafted healthy cions to diseased plants and found that mottling and flecking occurred in the cion shoots. Viruses being little understood at that time, this transmission apparently did not suggest a virus disease to Peltier. Lamkey (4), who followed Peltier in the work at Illinois, reached no conclusion as to the cause of yellows, but in his discussion he suggested that it might possibly be caused by a virus. Fukushi (3) included carnation mosaic in a list of virus diseases found in Japan in 1932, but described no symptoms and cited no evidence that the disease was caused by a virus. In 1937 Smith (7) referred to a disease of carnations found in England as a suspected virus disease, in which the leaves are mottled and the flowers are broken. Smith considered that further investigation was necessary to definitely establish its cause. Asuyama (2), in 1938, reviewing recently reported diseases of cultivated plants in Japan, summarized a paper on mosaic disease of the carnation by Abe (1), another Japanese worker. He briefly described symptoms of leaf mottling and flower breaking closely resembling those associated with carnation mosaic in the United States and referred to the pathogen as a virus, but cited no evidence to prove that the disease actually was caused by a virus. In 1939, Pirone (6) listed carnation mosaic as a virus disease, but gave no evidence that would indicate the cause to be of virus nature.

From these accounts, chiefly mere mentions or imperfect descriptions, one cannot be certain that the various maladies listed are the same or that any one of them is identical with the disease referred to in this paper as carnation mosaic. Also, none of these accounts gives conclusive experimental evidence that any carnation trouble referred to is caused by a virus.

[1] Appreciation is expressed to Dr. Leo R. Tehon for his helpful suggestions given during the course of this study and in the preparation of the manuscript; also, acknowledgement is made to Dr. R. R. Kudo, Department of Zoology, University of Illinois, for translating that portion of Asuyama's article dealing with carnation mosaic.

SYMPTOMS

Plants affected with carnation mosaic show mottling and flecking of leaves and stems and breaking and distortion of flowers; also, the general vigor and productiveness of diseased plants are reduced. Young leaves of affected plants show mottling of light- and dark-green patches or streaks that run parallel with the midribs, causing the affected leaves to appear lighter green than those of healthy plants (Fig. 1). Older leaves of affected

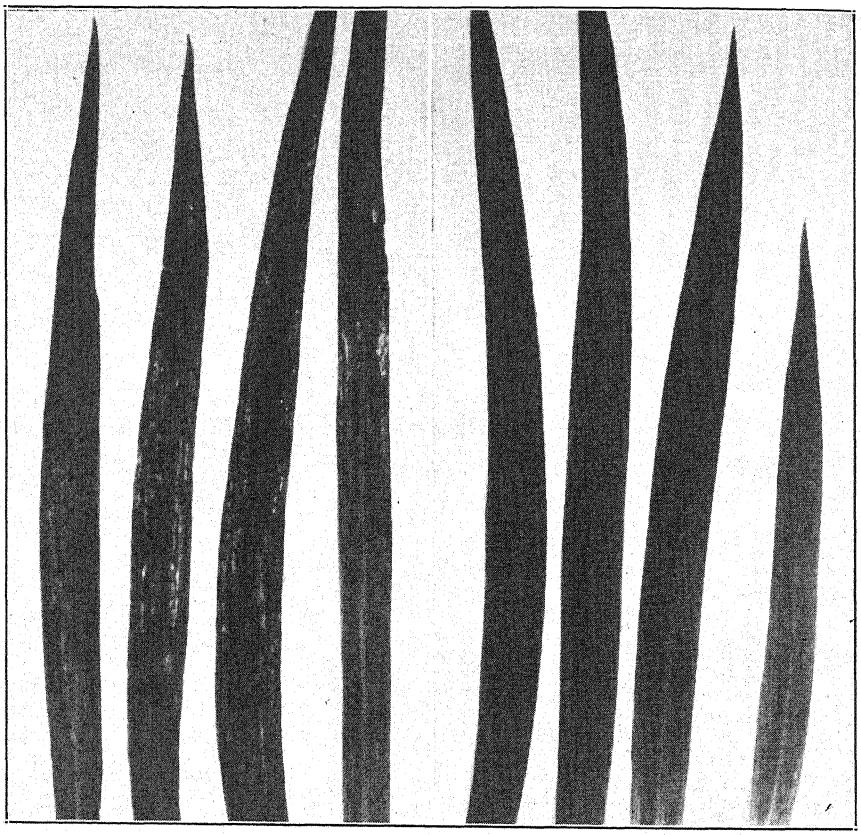


FIG. 1. Carnation mosaic. At the left, leaves in an advanced stage of infection showing mottling, flecking and streaking; at the right, normal leaves. Photographed with transmitted light.

plants show whitish, sunken, elongated flecks or streaks, which eventually can become reddish, purplish or brown. Sometimes severely affected leaves are blighted. Also, stems of diseased plants frequently show whitish flecks or streaks similar to those shown on leaves.

In the colored varieties, flower breaking accompanies mottling and flecking of leaves (Fig. 2). Broken flowers show white or light-colored streaks that parallel the veins of the petals, fanning out from the base toward the tip. In the white-flowered varieties, the typical leaf symptoms appear, but

the flowers do not show breaking because they are white. Regardless of color, flowers of affected plants usually are distorted and of poor quality.

### TRANSMISSION STUDIES

Repeated attempts to associate a fungus or bacterium with the sunken necrotic flecks in the older leaves of affected plants by microscopic examination and culture studies have failed, but the infective principle has been successfully transmitted from diseased to healthy plants by grafting and has been carried repeatedly from one crop of plants to the next in cuttings taken

FIG. 2. Carnation mosaic. At the left, an opening flower bud of King Cardinal variety showing broken or white-streaked petals as a result of inoculation by grafting; at the right, a normal flower of the same variety.

from diseased plants. In a preliminary experiment, a total of 9 cions of healthy King Cardinal carnations were grafted to 4 diseased plants of the Pink Treasure variety. All cions have developed branches with typically mottled and flecked leaves, and all that have flowered have produced broken and distorted flowers.

In a more extensive grafting experiment, 315 plants were propagated from carefully selected healthy plants of the King Cardinal variety. To 215 of these healthy plants, cions from diseased plants of the King Cardinal and Pink Treasure varieties were grafted. The remaining 100 plants, consisting of 2 or 3 plants of each clone of healthy plants, to which no cions were grafted, were retained as checks. Out of the first series of 110 grafted plants, 91 successful grafts were obtained, and in the second series of 105 grafted plants, 101 were obtained. Although this experiment still is in

progress, the results obtained thus far are very indicative. In the first series, 90 plants out of 91 show marked symptoms of mottling and flecking of leaves, and of 32 plants that have flowered all yielded typically broken and distorted flowers. In the second series, which was started somewhat later than the first series, 78 plants out of a total of 101 show symptoms of leaf mottling and flecking, and of 20 that have flowered all produced broken flowers. None of the 100 check plants shows symptoms of the disease. The results of these grafting experiments show that the infective principle can be transmitted from stock to cion and from cion to stock by grafting, providing good evidence that the disease is caused by a virus.

Thus far the virus has not been mechanically transmitted to healthy plants. An experiment designed to transmit the virus from diseased to healthy plants by alternate pinching or topping of diseased and healthy plants has given negative results. Likewise, an experiment designed to induce the disease in healthy plants by rubbing the leaves with juice from diseased plants, after the leaves were dusted with carborundum powder, has been unsuccessful.

Observations made in commercial plantings of carnations indicate that disease-free lots of plants can become infected when grown near mosaic plants in the same greenhouse or field plot. Results of an experiment conducted last year gave similar indications. In this experiment, 300 young plants of King Cardinal carnations were purchased from a grower and were separated at random into two lots. One lot of 200 plants was grown in a commercial field plot during the summer, adjacent to rows of other carnations containing a high percentage of mosaic, while the other lot of 100 plants was grown in the greenhouse, where it was isolated from plants known to be diseased. To a considerable extent plants grown in the greenhouse were protected from insects, while no attempt was made to control insects in the field. In the fall plants grown in the field plot were brought to the greenhouse and planted in a bed adjacent to those grown in the greenhouse all summer for comparison. By the following spring 30 per cent of the field-grown plants were mosaicked, showing typical symptoms of leaf mottling and flower breaking, while only 6 per cent of those grown in the greenhouse showed symptoms of the disease. Since all attempts to transmit the virus from diseased to healthy plants by mechanical means have failed, it is strongly suspected that insects are vectors. Insects as vectors would, of course, explain the rapid spread of the virus from infected to healthy plants in commercial plantings.

## SUMMARY

Carnation mosaic has been observed frequently in Illinois greenhouses and similar, if not identical, carnation troubles have been reported previously from the United States, Europe, and Asia.

Leaves and stems of affected plants are mottled, streaked and flecked and flowers of colored varieties are broken.

Results of experiments and observations indicate that carnation mosaic is caused by a virus, evidence which earlier workers have not presented. The virus can be transmitted readily from diseased to healthy plants by grafting, it can be carried from one crop to the next in cuttings taken from infected plants for propagation, and in the field it is spread from diseased to healthy plants, possibly by insects.

SECTION OF APPLIED BOTANY AND PLANT PATHOLOGY,
ILLINOIS NATURAL HISTORY SURVEY, URBANA, ILLINOIS.

LITERATURE CITED

1. ABE, T. Mosaic disease of the carnation. Zissai-Engei 23: 432–433. 1937.
2. ASUYAMA, H. New diseases and pathogenes reported recently on the cultivated plants in Japan—IV. Ann. Phytopath. Soc. of Japan 7: 231–236. 1938.
3. FUKUSHI, T. A contribution to our knowledge of virus diseases of plants in Japan. Trans. Sapporo Nat. Hist. Soc. 12: 130–141. 1932.
4. LAMKEY, E. M. R. Carnation yellows. Amer. Florist 48: 508–510. 1917.
5. PELTIER, GEO. L. Carnation diseases. Amer. Florist 46: 725–726. 1916.
6. PIRONE, P. P. Diseases of ornamental plants. New Jersey Agr. Exp. Stat. Circ. 385: 71. 1939.
7. SMITH, K. M. A textbook of plant virus diseases. P. Blakiston's Son & Co. (Philadelphia). Pp. 554–555. 1937.

# DIPLODIA PINEA AND SPHAEROPSIS MALORUM ON SOFT PINES

ALMA M. WATERMAN

(Accepted for publication March 19, 1943)

Occasional reports have been made of the occurrence of species of *Sphaeropsis* on cankers or dead areas on trunks or branches of eastern white pine (*Pinus strobus* L.), Himalayan pine (*P. griffithii* McClell.), and limber pine (*P. flexilis* James). In some cases the organism has been reported as *Sphaeropsis ellisii* Sacc. (3, 4), which has large brown continuous, or rarely one-septate, spores produced in pycnidia. The fruiting bodies and spores correspond to those of *Diplodia pinea* (Desm.) Kickx of which *S. ellisii* is a synonym (13). This fungus also has been reported by Slagg and Wright (11) as the cause of the death of seedlings of Colorado piñon pine (*P. cembroides* var. *edulis* (Engelm.) Voss). Another species of *Sphaeropsis* with spores smaller than those of *D. pinea* and more closely resembling *S. malorum* Pk., the pycnidial stage of *Physalospora obtusa* (Schw.) Cke., has been found on dead areas on branches or trunks of eastern white pine. Very little is known regarding the conditions under which these two fungi infect soft pines. In connection with a study of *D. pinea* on hard pines (13) a few observations on the occurrence of these fungi and a series of inoculations were made.

## DIPLODIA PINEA

Cankers caused by *Diplodia pinea* have been noted by the writer, particularly on eastern white pines growing in unfavorable locations, on poor soil, or densely shaded, or weakened by environmental agencies, or injured by mechanical means or by pruning. Crandall (2), however, found that *D. pinea* was the cause of a collar and root rot of white pine seedlings; and Pirone (9) obtained positive results from the inoculation of the stem of a 5-year-old white-pine seedling with mycelium from a single-spore culture of *D. pinea*. A report by Waterman and Miller (14) of a tip blight of a white pine growing in the vicinity of Austrian pines and Douglas firs heavily infected with *D. pinea* also indicates that under certain conditions the fungus is parasitic on eastern white pines. The parasitism of the fungus on Colorado piñon pine is evident from the investigations of Slagg and Wright (11).

In the study of *Diplodia pinea* on hard pines (13) three vigorously growing white pine trees in an experimental plot were inoculated by the writer with mycelium of the fungus from single-spore cultures isolated from infected needles of Austrian pine. The results were negative in all cases. Six inoculations on other white pines in the same plot were made with mycelium from single-spore cultures of *D. pinea* isolated from pycnidia on a canker around an old pruning wound on a white pine tree in Rhode Island. Two inoculations each were made by applying the inoculum in the following

ways: near the base of uninjured leaves of the new growth when the leaves were about one inch long; on leaf scars from which the newly developed leaves had just been removed; in wounds on twigs of the new growth made by cutting with a sterilized scalpel a small triangular section of bark under which the inoculum was inserted. After the inoculum was applied, the portion inoculated was wrapped with moist cotton and then covered with a strip of waxed paper firmly tied in place. The coverings were removed about 10 days after inoculation. Controls were made in the same way without the introduction of the inoculum. No positive results were obtained in any of the inoculations.

### SPHAEROPSIS MALORUM

In 1885 Peck (8, p. 98) reported a fungus on *Pinus strobus* as a new variety of *Diplodia pinea*, namely, *D. pinea corticola*. This variety is considered by Stevens (12) to be a synonym of *Sphaeropsis malorum*. His examination of Peck's specimen showed that the spores measured 22–24 μ × 11–13 μ, smaller than those of *D. pinea*, but within the range of *S. malorum*. Hesler (6) listed *P. strobus* as a host of *S. malorum* as indicated by collections only. The results of his cross inoculations of *P. strobus* with *S. malorum* isolated from apple were negative (5). Hedgcock (4) reported *S. ellisii* on *P. griffithii* (*P. excelsa* Wall.) from Pennsylvania. Notes by Dearness, accompanying the collection, indicate that the spores measured 20–25 μ × 7–8 μ, which he considered the same as those of *Macrophoma pinea* Passer. This latter fungus is described as having hyaline one-celled spores measuring 22.5 μ × 7.5 μ (10, p. 198). In an examination of the specimen by the writer a very few brown unicellular spores were found with spore measurements as given by Dearness and corresponding with spores of *S. malorum*. The occurrence of *S. malorum* causing a disease of Himalayan pine in Pennsylvania following winter injury was reported by York (15) and Jump (7). Cross inoculations proved that the fungus would infect through wounds made on the twigs of young apple trees and of 3-year-old white pines. Inoculations on 2- and 3-needle hard pines gave negative results.

In the present study a series of inoculations was made with *S. malorum* spores produced from single-ascospore cultures of *Physalospora obtusa* isolated by Ayers (1) from apple twigs in Massachusetts. Young healthy trees of *Pinus strobus* and of the hard pines, *P. nigra* Arnold, *P. sylvestris* L., and *P. resinosa* Ait., growing in the experimental plot, were inoculated with crushed pycnidia containing mature spores. The methods of applying the inoculum to the parts of the trees indicated in table 1 were the same as in the inoculations of *P. strobus* with *Diplodia pinea* mentioned above. From the thirty inoculations the one positive infection occurred on *P. sylvestris* around the base of a dead twig stub immediately adjacent to and partially included in the wound into which the inoculum was inserted. No canker formed on the living branch and the fungus was reisolated only from the

base of the dead twig stub. In all other cases the inoculated wounds healed over, as did those of the controls.

TABLE 1.—*Results of inoculations of species of Pinus with Physalospora obtusa isolated from apple twigs*

| Part inoculated | Number of inoculations and infections on species of *Pinus* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *nigra* | | *sylvestris* | | *strobus* | | *resinosa* | |
| | Inoculations | Infections | Inoculations | Infections | Inoculations | Infections | Inoculations | Infections |
| Buds uninjured | 3 | 0 | 3 | 0 | 0 | 0 | 2 | 0 |
| Leaves uninjured | 1 | 0 | 1 | 0 | 1 | 0 | 2 | 0 |
| Leaf scars | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| Twig wounded | 2 | 0 | 4 | 1 | 3 | 0 | 4 | 0 |

## SUMMARY

The results of these observations and inoculations indicate that neither *Diplodia pinea* nor *Sphaeropsis malorum* is parasitic upon the leaves and twigs of the new growth on young vigorously growing white pines. Both fungi, however, occasionally may be contributing factors in the unhealthy condition of soft pines that have been weakened primarily by other agencies. Improved growing conditions and the prevention or protection of wounds will lessen materially the possibility of infection.

DIVISION OF FOREST PATHOLOGY, BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING, AGRICULTURAL RESEARCH ADMINISTRATION,
U. S. DEPARTMENT OF AGRICULTURE, IN COOPERATION WITH
OSBORN BOTANICAL LABORATORY,
YALE UNIVERSITY, NEW HAVEN, CONN.

## LITERATURE CITED

1. AYERS, T. T. A recent collection of the ascigerous stage of *Physalospora obtusa* (Schw.) Cooke in Massachusetts. Mycologia 30: 598–599. 1938.
2. CRANDALL, B. S. A root and collar disease of pine seedlings caused by *Sphaeropsis ellisii*. Phytopath. 28: 227–229. 1938.
3. HAHN, G. G. Pathological and mycological notes on fungi observed on white pine. Blister Rust Control Conf. Rpt. Proc. 17 (1931): 49–51.
4. HEDGCOCK, G. G. Notes on the distribution of some fungi associated with diseases of conifers. Plant Dis. Rptr. (U. S.) 16: 28–42. 1932.
5. HESLER, L. R. Biological strains of *Sphaeropsis malorum*. (Abstract) Phytopath. 4: 45. 1914.
6. ———. Black rot, leaf spot, and canker of pomaceous fruits. Cornell Univ. Agr. Exp. Stat. Bull. 379: 49–148. 1916.
7. JUMP, J. A. Further notes on the disease of Himalayan pines. Morris Arboretum Bull. 1: 97–98. 1937.
8. PECK, C. H. Report of the New York State Botanist. New York State Museum Ann. Rpt. 38: 77–138. 1885.
9. [PIRONE, P. P.] *Sphaeropsis ellisii* on conifers. New Jersey Agr. Exp. Stat. Nursery Disease Notes 10: 43–46. 1938.
10. SACCARDO, P. A. Sylloge fungorum. Vol. 10. 964 pp. 1892.

11. SLAGG, C. M., and E. WRIGHT. Diplodia epidemic in conifer seedbeds. (Abstract) Phytopath. 32: 16–17. 1942.
12. STEVENS, N. E. Two apple black rot fungi in the United States. Mycologia 25: 536–548. 1933.
13. WATERMAN, A. M. *Diplodia pinea*, the cause of a disease of hard pines. Phytopath. ———. ———.
14. ———, and J. A. MILLER. A die-back of Douglas fir. Phytopath. 26: 804–805. 1936.
15. YORK, H. H. Diseases of the Himalayan pine. Morris Arboretum Bull. 1: 34–35. 1936.

# SPHAEROTHECA HUMULI VAR. FULIGINEA ON DELPHINIUM IN CALIFORNIA

KENNETH F. BAKER

(Accepted for publication March 17, 1943)

The presence in California of *Sphaerotheca humuli* (DC.) Burr. var. *fuliginea* (Schlecht.) Salmon on *Delphinium* spp.,[1] in addition to the ubiquitous *Erysiphe polygoni* DC., is potentially significant in a breeding program (3) for resistance to this disease.

Several plants of *Delphinium amabile* Tidestr. in the university glasshouses, West Los Angeles, were observed in February, 1942, to have many perithecia of the powdery mildew, *Sphaerotheca humuli* var. *fuliginea,* which may have been brought in on various transplanted native *Delphinium* spp. present at the time. Although no published record of this fungus on *Delphinium* in North America has been found, specimens examined in the University of California herbarium at Berkeley showed the following collection of this fungus under the label *S. humuli:* Berkeley, Alameda Co., Calif., on cultivated *D. consolida* L., July, 1924 (H. E. Parks 2759); Alton, Humboldt Co., Calif., on *D. trolliifolium* Gray, April 24, 1927 (J. P. Tracy 7950); Middle Yager Creek, Humboldt Co., Calif., on *D. trolliifolium,* July 6, 1930 (J. P. Tracy 8857); Coyote Peak, Bald Hills, Humboldt Co., Calif., on *D. trolliifolium,* August 6, 1933 (J. P. Tracy 12959). The Berkeley collection was made in the university botanical garden and carried the notation, "Destructive on this host"[2]; since this area has been rather well botanized it is surprising that there are no known later collections of the parasite there. Mr. J. P. Tracy stated[3] that the mildew ". . . on the wild *Delphinium trolliifolium* is frequent and often damages the host severely" in Humboldt County; he had no record of it on other wild species there. The Plant Disease Survey[4] had no additional records of this mildew on delphinium.

It is evident that the pathogen has been established on native delphiniums in northwestern California for at least 16 years, and was present in the state even earlier. Since other specimens (J. P. Tracy 8886, 9672, 13077½, and H. E. Parks 5102) on cultivated perennial delphinium and rocket larkspur from Humboldt County proved to be *Erysiphe polygoni,* it is uncertain whether the second mildew is established on cultivated varieties in that region.

Study of the various specimens showed several characteristics that allow rapid identification of *Sphaerotheca humuli* var. *fuliginea* under a dissecting microscope of 72 × magnification, using lateral illumination. The cells

[1] Several specimens were kindly made available for study by L. Bonar and G. A. L. Mehlquist of the University of California, and by J. P. Tracy, Eureka, California.

[2] H. E. Parks, in correspondence dated February 6, 1943, stated "The fine leaves were well covered with perithecia. . . ." Apparently exsiccati of this collection are fragmentary and relatively rare. Identification of the host was confirmed by G. A. L. Mehlquist.

[3] Letter dated January 30, 1943.

[4] Letter from J. A. Stevenson, dated February 6, 1943.

of the perithecial wall are large and decidedly convex, with the cell sutures rather deeply indented. By contrast, the walls of *Erysiphe polygoni* are composed of smaller cells which are relatively smooth or occasionally concave (*i.e.*, with the central portion of the cell collapsed, a condition apparently most common on immature perithecia). The walls of *E. polygoni* are also darker than those of *S. humuli* var. *fuliginea* and are usually more refractive. In all of the material examined the perithecia of *E. polygoni* were more or less embedded in aerial mycelium and conidiophores, but the other mildew had scant mycelial development; this agrees with the condition described by Blumer (1) for *S. delphinii* Karsten. The conidiophores projecting from the folded margins of leaves, examined as above, showed that conidia of *E. polygoni* were borne singly whereas those of *S. humuli* var. *fuliginea* were borne in chains, a distinction well illustrated by figures 2b and 2d of Brodie and Neufeld (2).

Examination under the compound microscope of many samples of perithecia from a large number of plants of the *Delphinium elatum* group (collected Aug. 10, 1942, in a field planting in West Los Angeles), indicated that the mildew was entirely *Erysiphe polygoni.* After the external structural differences of the perithecia were appreciated, reexamination of the material under the dissecting microscope disclosed small colonies of *Sphaerotheca humili* var. *fuliginea,* as well. Identification was confirmed in all cases by examination under high magnification. Because the new parasite is not so vigorous in its development as *E. polygoni,* it was overlooked by the usual methods of examination. This collection demonstrated that the fungus can become established under outdoor conditions in Southern California.

The new pathogen was found to have perithecia 60–89 μ (average 76.3 μ) in diameter, with conspicuous wall cells 13–28 μ (average 21.3 μ) in width. The asci were 46–88 × 43–71 μ (average 63.7 × 56.8 μ) and the eight ascospores were 13–26 × 13–18 μ (average 18.1 × 14.5 μ). The perithecial diameter is larger than that given in Salmon's monograph (5) for *Sphaerotheca humuli* var. *fuliginea* but within the limits of specimens later (6) referred to this variety; the measurement likewise fits *S. fuliginea* (Schlecht.) Salmon (*sic*) of Blumer (1) but is larger than *S. delphinii* which he separates from it. The size and conspicuousness of the perithecial wall-cells, the recognized criterion of separation of *S. humuli* and its variety *fuliginea,* place the fungus in the latter category, although the size is somewhat less than that reported by Blumer (1) or Salmon (5). The size of the asci and ascospores is not sufficiently distinct to aid in separation of the two fungi.

There apparently are no reports of *Sphaerotheca humuli* on Ranunculaceae but the variety *fuliginea* has been reported on *Delphinium grandiflorum* L. (1) in Siberia, *Paeonia anomala* L. (1) in Russia, *Thalictrum alpinum* L. (1, 5) in Norway and Sweden, *T. minus* L. (1) and *T. simplex* L. (1, 5) in Siberia, and *Trollius europaeus* L. (1) in Italy. *Caltha palustris* L., listed as a possible host in Salmon's monograph (5), was later (6) deleted.

*Thalictrum angustifolium* L., listed by Oudemans (4), is obviously an error, since the sources cited give no such host.

It is possible that *Sphaerotheca humuli* var. *fuliginea* is established on delphiniums in areas other than California without having been reported. The tolerance of *Erysiphe polygoni* to low humidity (2, 7) may explain the fact that it is more abundant than *S. humuli* var. *fuliginea* on delphiniums in California. However, the new mildew might be damaging in more humid sections.

SUMMARY

*Sphaerotheca humuli* var. *fuliginea* has been present on cultivated and wild *Delphinium* spp. in California for at least 19 years. No published records have been found of the occurrence of this fungus on *Delphinium* or other Ranunculaceae in North America, but it has been reported elsewhere on several representatives of the family, mostly in northern Europe and Siberia.

Although this parasite apparently is rather rare on present commercial delphiniums it is a potential threat to new types which may be developed. The determination of the present distribution of the fungus is, therefore, of interest. This is facilitated by a method for rapid examination of quantities of mildew material to determine whether *Sphaerotheca humuli* var. *fuliginea* is present with the ubiquitous *Erysiphe polygoni*.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
LOS ANGELES, CALIFORNIA

LITERATURE CITED

1. BLUMER, S. Die Erysiphaceen Mitteleuropas mit besonderer Berücksichtigung der Schweiz. Beitr. Kryptogamenfl. Schweiz. 7: 1–483. Gebr. Fretz (Zürich). 1933.
2. BRODIE, H. J., and C. C. NEUFELD. The development and structure of the conidia of *Erysiphe polygoni* DC. and their germination at low humidity. Canad. Jour. Res., C, 20: 41–61. 1942.
3. MEHLQUIST, G. A. L. The reaction of thirteen California species of *Delphinium* to powdery mildew. Proc. Amer. Soc. Hort. Sci. 39: 411–413. 1941.
4. OUDEMANS, C. A. J. A. Enumeratio systematica fungorum 3: 172. Martin Nijhoff (The Hague). 1921.
5. SALMON, E. S. A monograph of the Erysiphaceae. Torr. Bot. Club. Mem. 9: 1–292. 1900.
6. ————. Supplementary notes on the Erysiphaceae. Bull. Torr. Bot. Club 29: 1–22, 83–109, 181–210, 302–316, 647–649. 1902.
7. YARWOOD, C. E. The tolerance of *Erysiphe polygoni* and certain other powdery mildews to low humidity. Phytopath. 26: 845–859. 1936.

## PHYTOPATHOLOGICAL NOTE

*Mercury Compounds Applied to Banana Plants in the Field.*[1]—Mercury compounds kill *Fusarium oxysporum cubense*[2] and, therefore, prevent its spread[3] in low concentrations in the soil. A series of studies was made in the field to determine the effect of the mercury compounds on the banana plant and on the Panama-disease organism. In these experiments the following mercury compounds were used: DuBay 1155–HH, 5 per cent ethyl mercury iodide (Bayer-Semesan Co.); Hortosan Potato Dip, 8 per cent nitrophenol-mercurihydroxide and chlorophenolmercurihydroxide (Imperial Chemical Industries Ltd.); mercurous chloride; and mercuric chloride.

In the first experiment 3 banana plants were selected and 2 plots 2′ × 2′ dug 2′ from each plant, close together. The soil from each plot to a depth of 6 inches was removed to a canvas. Hortosan was mixed with the soil of one plot from each tree at the rate of 2 oz., 4 oz., and 8 oz., per sq. ft., respectively, and replaced. The nontreated soil was replaced on the check plots. At the end of 1 month the roots at the edges of the 8-oz. plot had turned up and were hypertrophied. At the end of 10 weeks the soil was again removed from all the plots and the roots separated and weighed. The roots from the Hortosan 2-oz., 4-oz., and 8-oz. plots weighed 14 g., 8 g., and 0 g. respectively, while the checks weighed 38 g., 28 g., and 59 g. The soil was then replaced. Five months later the roots from the Hortosan plots totalled 83.4 g. and the checks 79.7 g. No root injury was observed. Five months after the beginning of this experiment the soil was tested for the growth of *Fusarium oxysporum cubense*. Tubes were half-filled with soil from the treated and nontreated areas, autoclaved, planted with the fungus, and the growth rate compared.[4] The soil from the Hortosan plots permitted no growth, while the checks produced normal growth. At 7 months the same results were obtained. After the Hortosan had weathered in the soil for 9 months the *Fusarium* growth readings were 1.5 cm., 0.0 cm., and 0.0 cm., with the checks normal at 3.1 cm., 3.1 cm., and 4.0 cm. After 11 months the 2-oz. per sq. ft. treatment permitted a normal growth of the Panama-disease fungus, but the 4-oz. and 8-oz. treatments gave reduced growth.

In the second experiment, at the time of planting, the mercury compounds were mixed with the soil around the plant within a radius of 2 feet. The application of 2 oz. per stool of Hortosan or 1 oz. of DuBay, mercurous chloride, or mercuric chloride at the time of planting apparently gave no injury. A 1-oz. per plant treatment of Hortosan inhibited the growth of

[1] Acknowledgment is due the Jamaica Banana Producers' Association for support in this research.

[2] Meredith, C. H. The effect of chemicals on *Fusarium oxysporum cubense* growing in the soil. Phytopath. 32: 182–184. 1942.

[3] Meredith, C. H. The effect of soil and chemical mixtures on the growth of *Fusarium oxysporum cubense*. Phytopath. (in the press).

[4] Meredith, C. H. The growth of *Fusarium oxysporum cubense* in the soil. Phytopath. 31: 91–93. 1941.

*Fusarium oxysporum cubense* for 3 months on acid soil and 2 months on neutral soil. In plots on 2 different soils the 2-oz. treatment of Hortosan has given a greater growth of the banana plant than the growth on the non-treated soil.

In the third experiment Hortosan potato dip was spread over the area covered by the roots of 3 different plots. There were 2 stools in each plot. The Hortosan was applied at the rate of 3 oz. per 100 sq. ft., 6 oz. per 100 sq. ft., and 12 oz. per 100 sq. ft. Soil samples were taken and growth readings made with *Fusarium oxysporum cubense*. The readings were not consistent, perhaps due to the fact that the mercury compound was sprinkled on the surface rather than mixed with the soil, as in the other experiments. There was no injury to the banana plants observed for 8 months after this treatment.

The banana plant apparently can withstand higher concentrations of mercury compounds than *Fusarium oxysporum cubense*. The Hortosan potato dip was observed to stop the growth of the Panama-disease organism in the soil after 9 months weathering. The Hortosan was more effective in acid soils than in neutral.—CLIFFORD H. MEREDITH, Glenleigh Laboratory, Highgate P. O., Jamaica, B. W. I.

# COMPARATIVE SYMPTOMATOLOGY OF PSOROSIS VARIETIES ON CITRUS IN CALIFORNIA[1]

H. S. FAWCETT[2] AND A. A. BITANCOURT[3]

(Accepted for publication March 3, 1943)

Studies and observations during the last few years have shown that a number of symptoms on citrus, previously considered those of totally different diseases, can now be connected with the virus disease psorosis through certain features of these symptoms on both bark and leaves (5, 6, 10, 11). The use of the term *psorosis*[4] is, therefore, expanded to cover a group of disorders now thought due to varieties of one virus. These disorders include psorosis A, psorosis B, concave gum, blind pocket, crinkly leaf, and others of minor importance, to be mentioned later. The last three are, accordingly, now designated as varieties of psorosis and called "concave-gum psorosis," "blind-pocket psorosis," and "crinkly-leaf psorosis." Since transmission of these varieties of psorosis has been possible only through use of living cell tissue, the present grouping is made on the basis of certain characters and symptoms that are common to all.

The young-leaf symptoms, although somewhat variable within each variety of psorosis, are those most consistently similar throughout the entire group. Another feature, fundamentally similar in the different varieties of psorosis when viewed in broad aspect, is an alteration near the cambium in the primary lesion of the wood, the varieties differing chiefly in the extent of the alteration produced.

It is the object of this paper to describe the gross external and internal characters of the different varieties of psorosis in some detail and to point out the common features as well as the differences. Some other, minor effects, occurring on citrus in California, possibly due to a related virus or viruses, are described briefly.

## YOUNG-LEAF SYMPTOMS OF ALL THE PSOROSIS VARIETIES

Since the young-leaf symptom (3, 4) is common to all the psorosis varieties, and since its similarity in the varieties seems to tie them together into one group, this character will be described before differentiating the varieties.

Small, elongated areas, white to yellowish or distinctly lighter to slightly paler green than the rest of the leaf blade, occur on the leaves in the region of the veinlets. These white to light-green areas are usually about 1 to 3

[1] Paper No. 488, University of California Citrus Experiment Station, Riverside, California.

[2] Professor of Plant Pathology and Plant Pathologist in the Experiment Station, University of California.

[3] Plant Pathologist, Instituto Biologico, São Paulo, Brazil. On John Simon Guggenheim Memorial Foundation Fellowship for research at the University of California Citrus Experiment Station, Riverside, 1942.

[4] *Psora* = a cutaneous disease, the itch, scabies; *osis* = state or condition. The name was first suggested by Swingle and Webber (14).

mm. long and ¼ to 1 mm. broad, the long axis being parallel with the main side veins (Fig. 1). They may be very numerous and scattered over the entire blade, or they may occur only on certain limited portions of the leaf blade. Sometimes only a small percentage of leaves of suitable growth

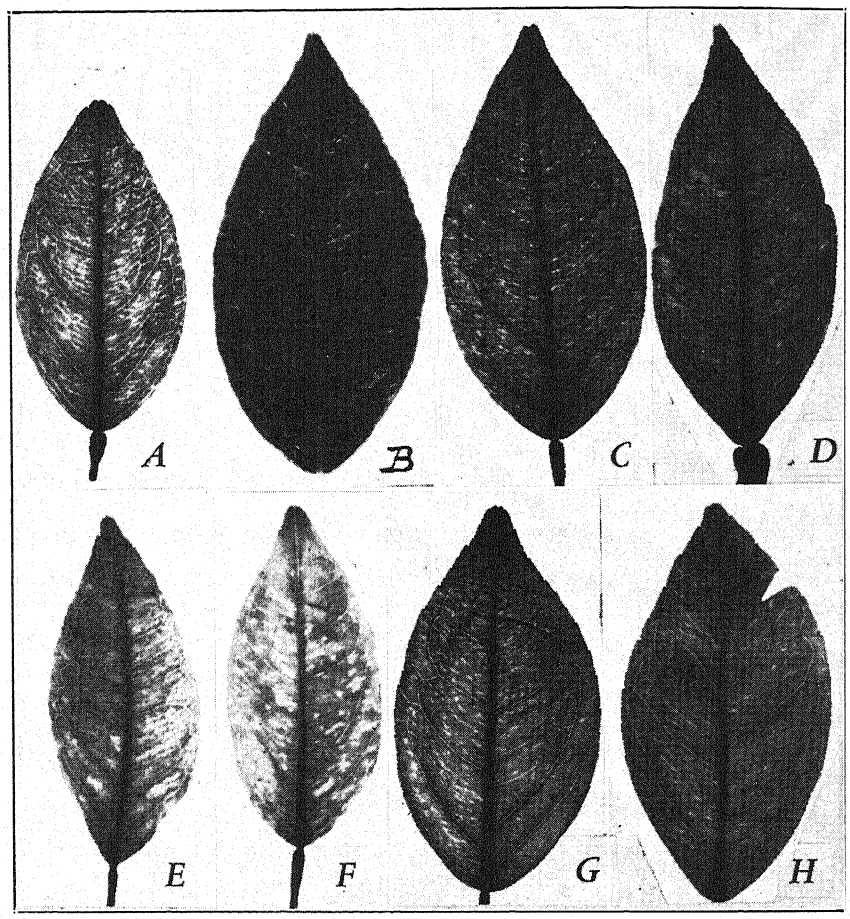



FIG. 1. Some of the most common aspects of young-leaf symptoms of psorosis, on orange leaves. Even greater variations in size and distribution of the cleared areas than here shown can be found in connection with each of the varieties of psorosis. The young-leaf symptoms can therefore not be used to distinguish the varieties. These leaves were from trees affected as follows: A and B. Psorosis A. C and D. Psorosis B. E and F. Blind-pocket psorosis. G. Crinkly-leaf psorosis. H. Concave-gum psorosis. All × about ¾.

shows these symptoms, but at other times a great majority of the leaves will show them. On young, tender leaves, one-quarter to one-half grown, the veinlets, as well as tissue adjacent to or between them, may show faint to pronounced clearing. It is not uncommon for a leaf or two to show distinct symptoms, while other leaves of apparently similar age on the same twig or on adjacent twigs show no visible symptoms.

Often the small flecks are indistinct and fade out gradually at the margin or coalesce and form a large light-green area. Sometimes a clearing extending along the veins in the form of a band is the principal feature.

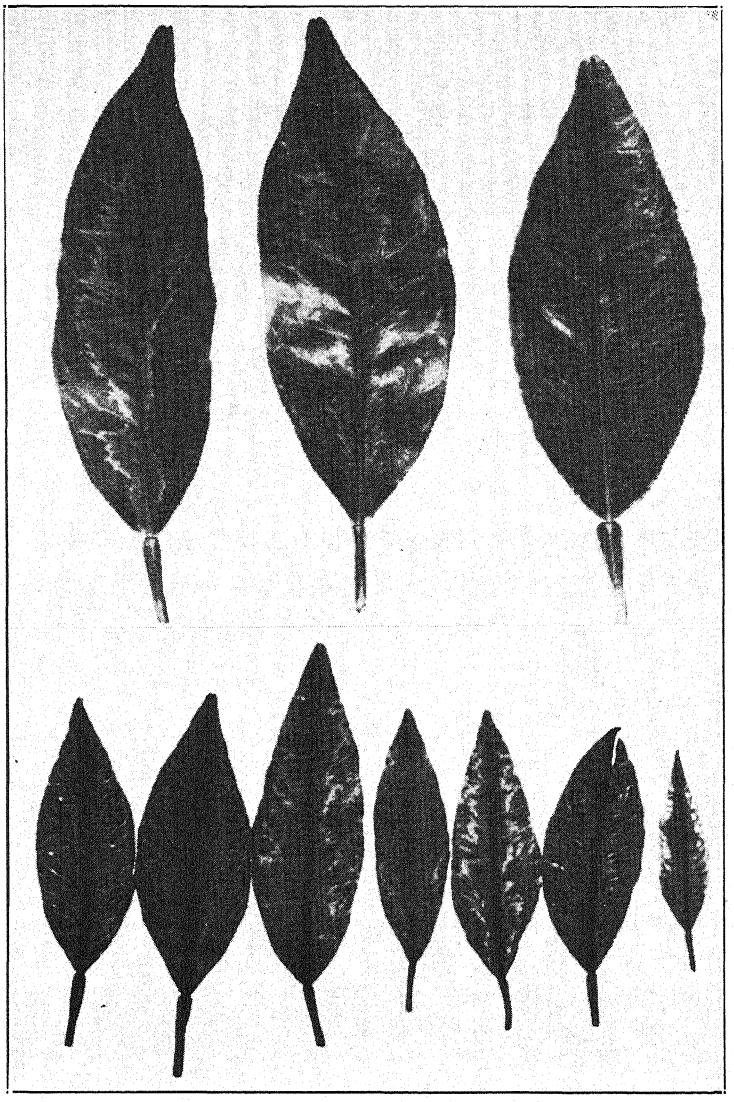


FIG. 2. The zonate or oak-leaf pattern of psorosis on young orange leaves, seen especially in the spring and most commonly on trees affected with concave-gum psorosis. Leaves in upper row about natural size; those in lower row × about ¾. Photographed by L. J. Klotz.

Frequently, the cleared region forms a distinct, characteristic pattern known as the zonate or oak-leaf pattern (Fig. 2). This is seen in the spring or early summer and is most commonly associated with the concave-gum pso-

rosis. At other times of the year, this pattern is not present even on trees affected with concave gum, and the young-leaf symptoms are then indistinguishable from those of the other psorosis varieties.

To see these symptoms most readily, the young quarter- to half-grown leaves should be shaded from the direct sun and viewed with strong transmitted light or with the light of the sky coming through the blade. The light-yellow to pale-green areas or flecks will show in contrast to the darker green of the rest of the blade (3, 4, 5, 6).

Caution is necessary to distinguish between psorosis symptoms and leaf injuries caused by thrips, red spider, small sharp hail stones, minute sand grains driven by wind, or the slight rubbing of the leaves against branches, all of which may produce markings similar in size to those of psorosis. If the leaf is turned over slowly in the direct sunlight, such injuries can often be detected and discounted.

As the leaves mature, the cleared areas or young-leaf symptoms caused by the psorosis disappear. On the mature leaves, other symptoms, such as circular spots, ring spots, concentric-ring spots, gum excrescence, blotches, crinkle, variegation, etc., may develop; but since these symptoms are not common to all the psorosis varieties, they are not described until later.

### PSOROSIS A

Psorosis A, which is caused by the virus *Citrivir psorosis* var. *vulgare*[5] (7, 8), is by far the most common variety of psorosis. Although slower to develop deleterious effects and less serious in its effect on a given individual tree, psorosis A is responsible for much more damage than is psorosis B.

The citrus species most severely affected by psorosis A, in respect to bark symptoms, are sweet orange, grapefruit, and tangerines, but other species show the leaf symptoms. In severity, psorosis A ranges from very mild to very severe. Some strains of the virus produce bark symptoms on sweet-orange trees within 6 years from the time of budding, while others may take 15 to 20 years. It is possible that some strains produce only leaf symptoms and never bark symptoms during the usual life of the tree. One form is found occasionally on the bark of sour orange, a variety usually resistant to bark lesions. How much of this variation is due to difference in virulence of strains and how much to differences in host resistance under different conditions, is not yet clear.

#### Bark Symptoms

The symptom expression on the bark usually begins either as small scales or flakes of outer bark (Fig. 3, B and C), with or without gum formation, or as aggregations of small, erumpent pustules (Fig. 3, A), under which the tissue is brown. The scales of outer bark are dry, irregular flakes, about $\frac{1}{12}$ to $\frac{1}{8}$ inch in thickness, which separate and peel away, exposing the tan- to

[5] *Citrivir*, from *citri* (Latin genitive of *citrus*) and *vir* (stem of *virus*) = virus of citrus; *psorosis* (Latin genitive of *psorosis*) = of the disease known as psorosis; *vulgare* = common.

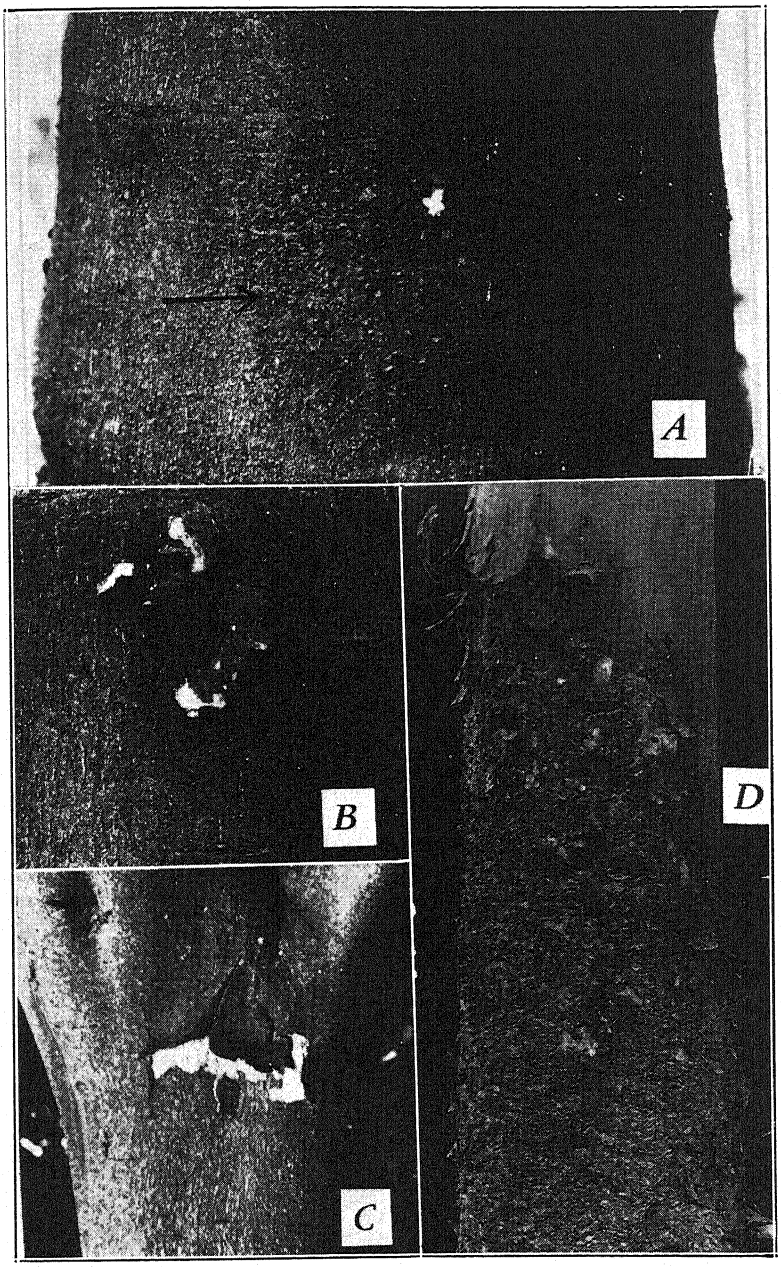



FIG. 3. Psorosis A. Different stages and forms of bark symptoms on trunks of orange trees. A. Early stage: aggregations of small, erumpent pustules—the pimply form in central portion. × about ¾. B. and C. Early stages of scaling. B, × about ¾; C, × about ½. D. Later stage of scaling. × about ½.

buff-colored surface of the live layer of bark underneath. These scales or pustules, as the case may be, usually occur first in localized areas on the bark of the trunk or limbs of trees 6 to 12 or more years of age (rarely earlier). As the scaling advances (Fig. 3, D), the deeper layers of bark are affected by irregular growth and by gum or gumlike deposits. Small or large amounts of gum often form, the amount depending upon weather and growth conditions and probably upon the strain of the virus (5). The rate of scaling varies greatly on different trees and probably represents the differential effects of strains.

The histological aspects of psorosis A in affected bark have been given in some detail by Webber and Fawcett (15) and by Fawcett (5). In brief, yellow-to-brown contents are first seen in some of the parenchyma cells of the phelloderm and of the primary cortex of the outer bark underlying the affected surface. Later, some of the groups of brown cells near the surface are cut off by a phellogen or cork-cambium layer producing phellem or cork cells toward the outside and phelloderm on the inside. These outside layers, cut off by the cork layer, die and form the dry scales or flakes of bark.

The increased phelloderm in diseased bark causes excessive thickening and frequently contains, in addition to the parenchyma, broken bands of enlarged stone cells. After the primary cortex has scaled off, the browning continues in the newly formed phelloderm and later extends into the living phloem. With further progress, the irregular contour of the cork cambium, which continues to produce an abnormal amount of phelloderm, creates pressures resulting in distortions within the underlying tissues. Microchemical tests of the brown contents in certain parenchyma cells, mentioned above, indicate that they may contain phenolic compounds, fatty substances, gum, and even ligninlike substances. Gum pockets involving only parenchyma tissue occur in thick phelloderm. In badly diseased tissue, the entire cortex and most of the phloem may be replaced by a group of wood cells surrounded by cambium.

## Wood Symptoms

*Primary Lesions.* Coincident with or following the development of the bark lesions, gum deposits are formed within and between layers of wood, more or less corresponding to seasonal and annular rings of growth. These layers of gum, since they comprise the first visible effects in the xylem region, are described as the primary lesions of the wood (Fig. 4). The continuous regions of gum are apparently formed by the dissolution of embryonic cells, usually involving only cells between rays. Cells bordering the gum regions may be filled with gum. Some cells of wood tissue produced at periods of growth when conditions do not favor the formation of gum regions, are normal or may contain lignified, thin-walled isodiametric cells with pits showing indistinct borders. Plugging of vessels, due to the accumulation of gum in the vicinity of perforated plates, is often seen in apparently normal wood between the gum layers before the secondary

lesions develop. The rate of wood growth becomes irregular in some psorosis lesions (15).

*Secondary Lesions.* A number of years after the inception of the bark lesions and the formation of the primary lesions of the wood containing gum regions, important changes take place in the deeper wood layers. These alterations do not usually begin until the lesions have become extensive, usually not before the trunk or limb is girdled by the bark lesions, with their accompanying primary lesions in the peripheral wood layers beneath.

In the beginning stage, these alterations, or secondary lesions, are seen

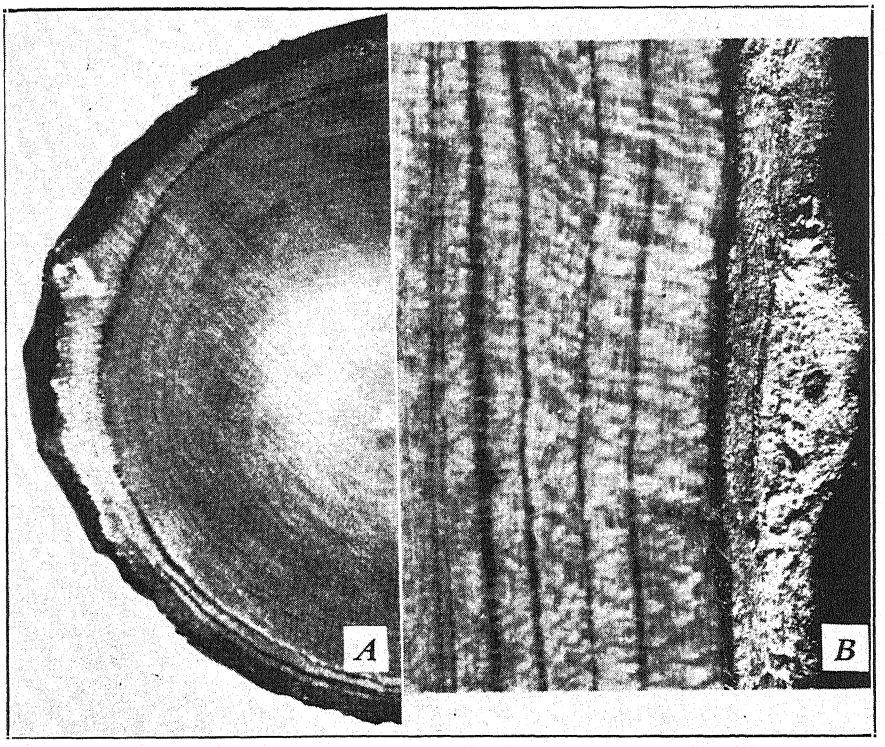



FIG. 4. Psorosis A. Wood symptoms: primary lesions. A. Transverse section of orange branch, showing overlapping gum layers at the periphery under a bark lesion. About natural size. B. Longitudinal radial section, showing successive gum layers under a bark lesion. × about 4.

mostly in the region of the axis of the wood rings and manifest themselves by extremely irregular discoloration of the wood, the outlines of which, in longitudinal sections, appear to be completely independent of the pattern of the wood elements, vessels, parenchyma, or medullary rays (Fig. 5, C and D). In transverse sections, however, it is often seen that the discoloration of the wood, although very irregular, is arranged in a way that suggests a relationship with the medullary rays (Fig. 5, A and B).

Two very distinct types of discolorations are generally apparent in transverse sections. The periphery of the discolored areas consists of an irregu-

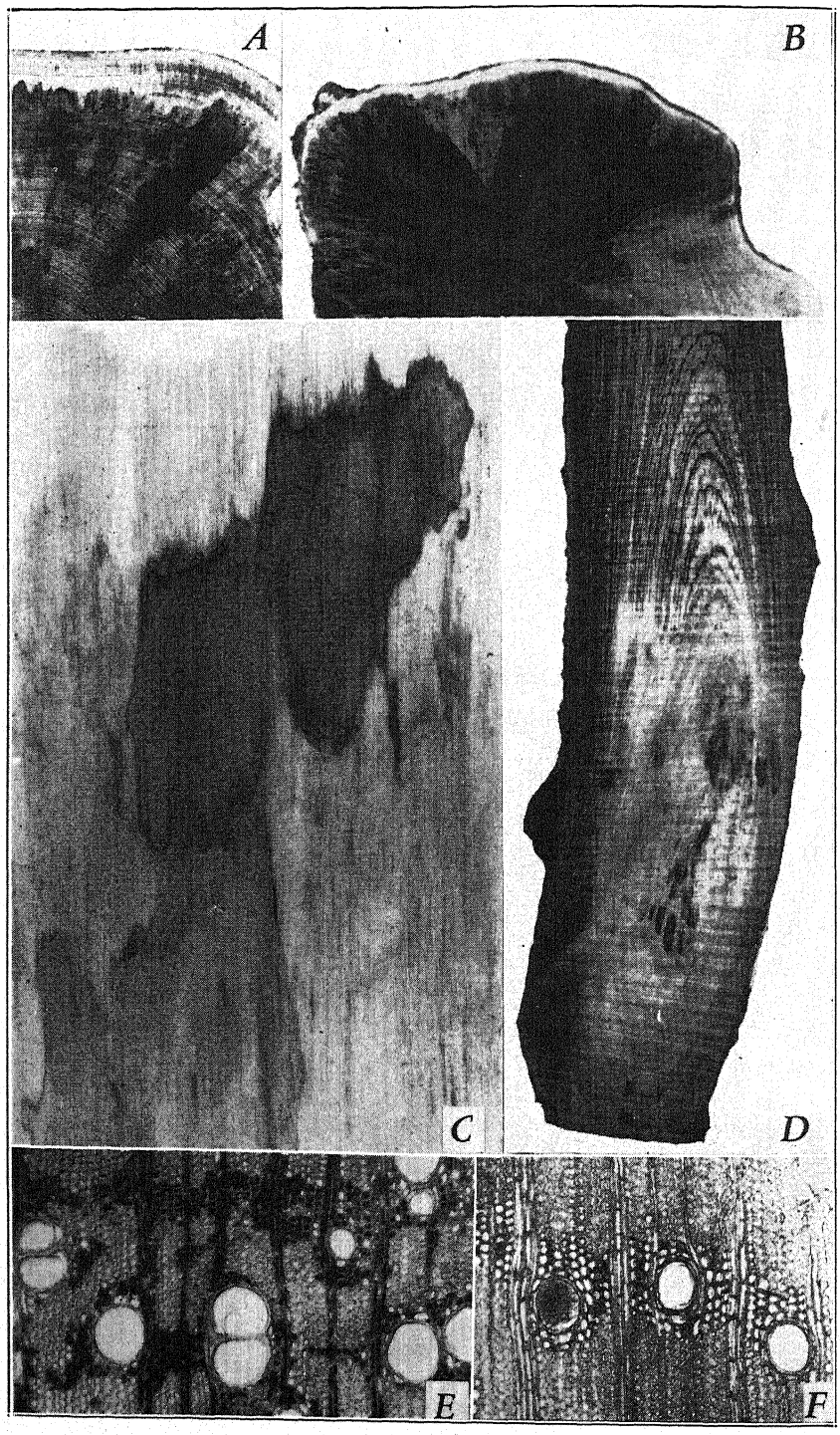



FIG. 5. Psorosis A. Wood symptoms: secondary lesions. A and B. Transverse sections of orange branches, showing wood discoloration well developed. × about $\frac{1}{2}$.

lar, narrow, well-defined, orange-brown or reddish-brown line. Within this well-marked limit, the wood is irregularly stained, the predominating colors being drab to brown. In portions of the lesion, however, the marginal orange-brown line may be very faint or even entirely lacking, and the wood discoloration may gradually fade into the normal wood color at the periphery of the lesion.

The difference between the two types of discolored wood is well emphasized by examination in the light of an ultraviolet lamp, in which both are distinctly fluorescent. The narrow, sinuous marginal line has an orange-colored fluorescence especially visible in fresh material. The central, irregularly discolored portion is light purple or lilac. The fluorescence may be almost completely absent in the most central parts of the discoloration and is extremely intense in the periphery, especially when the marginal line is absent. This suggests that the latter is formed later than the central discolored area, and that it might be the result of a wood reaction tending to seal off the altered wood from the still normal wood.

In very advanced cases, the wood alterations are more pronounced; this is usually due to the invasion of the wood by secondary wood parasites. These more pronounced alterations are no longer typical of psorosis, their characteristics varying according to the nature of the secondary parasites concerned. Ordinarily, such alterations are definitely connected with some injury, such as the pruning of a limb, in the region of the psorosis lesion. The upper surface of horizontal limbs is apparently a region of stress that is exposed to different natural or man-made injuries, which open up the way for wood-invading fungi.

The wood discolorations that have just been described are accompanied by the formation of gum or gumlike substances in the wood tissues. Part of the color is probably due to the gum itself. This gum is especially abundant in the peripheral orange-colored line, where it pervades all tissues, vessels, fibers, parenchyma, and medullary rays. If a tangential section of the wood in the region of this line is observed with a powerful hand lens, the medullary rays are seen to stand out distinctly as a result of their deeper orange coloration. This would seem to indicate that there is more gum formed in the medullary rays than in the other elements of the wood. This is not the case, however, because in transverse sections the medullary rays actually appear lighter in color than the other elements, especially the wood fibers. This difference in the aspect of the wood elements in the two kinds of sections is the result of viewing the gum-filled elements endwise in tangential sections and sidewise in radial and transverse sections. The orange-colored gum pervades, indistinctly, all the wood elements in the region of

C. Longitudinal radial section of an orange trunk, showing well-developed wood discoloration. × about $\frac{3}{4}$. D. Longitudinal section of orange branch treated with I–KI, showing starch present in the normal wood and a starch-free region surrounding the wood discoloration. × about $\frac{3}{4}$. E. Transverse section from normal wood, showing starch. × about 75. F. Wood from region near discolored wood, showing no starch and a vessel plugged with gum. All vessels had gum plugs (see accompanying paper [2], Fig. 1). × about 75.

the marginal line. In the central portions of the lesions, there seems to be less gum formed than in the marginal line, and the nature of the gum seems different, as shown by the differences in coloration when sections are stained with the usual gum reagents such as phloroglucin, orcinol, or Sudan IV. Both gums are insoluble in hot water, however.

When I–KI (iodine-potassium iodide) is applied to the secondary wood lesions, the reaction shows that there is very little or no starch in the discolored portion of the wood and, further, that starch is absent, or is very much reduced, in a more or less extensive region around the discolored portion, which is not otherwise differentiated from the normal wood (Fig. 5, D). This starch reaction establishes a usually well-defined boundary outside the stained portion and probably indicates the outer advancing margin of the altered wood. It will be seen later that the starchless area, which is undetectable in macroscopic appearance from the normal wood, will not allow the passage of water and therefore contributes to decline of the affected trees in the same way as the wood that has already become discolored (2, 12).

In microscopical transverse sections of normal wood treated with I–KI reagent, starch grains are chiefly distributed in three regions—in the medullary rays and in the metatracheal and paratracheal parenchyma (Fig. 5, E). In these same regions of the discolored portion of the wood, hardly any starch grains are seen, and there are only a very few in the contiguous region (Fig. 5, F), in which there is no macroscopical starch reaction with the I–KI test.[6] In this uncolored but starchless region, no gum can be detected macroscopically; but in transverse sections, and especially in longitudinal sections, it is seen that a sufficient amount of gum is formed in different parts of the vessels to explain the lack of passage of water in the experiment described in the succeeding paper (2). This gum probably originates from the starch (1), and its formation may, therefore, explain the decrease in the presence of starch.

Before the secondary symptoms of the wood are evident, the top growth and foliage of the tree are usually normal. After the secondary symptoms develop, the tree deteriorates rapidly, leaves become small, yellow, and few in number, and twigs begin to die back. The degree of decline is probably nearly proportional to the degree in which the starch-free discolored wood and its contiguous region of starch-free nondiscolored wood occupy the whole region of water-conducting tissue. It is possible, however, that other factors, such as nutrition or toxic products, may also play some part in deterioration of the tree (2).

## Leaf, Twig, and Fruit Symptoms

Young-leaf symptoms common to all the varieties of psorosis (Fig. 1) have been described earlier in this paper. Occasionally, in severe cases of psorosis A, the flecks or cleared areas on the leaf will persist for a while after the leaf begins to mature. Occasionally, also, circular spots similar to those

[6] The specimens examined were collected during the months of January to May.

described under psorosis B develop on mature leaves. Twig lesions like those described under psorosis B also develop with psorosis A, but much less frequently than with psorosis B; they usually are produced only by virulent strains of the virus. Fruit spots and sunken furrows or rings, such as those described under psorosis B, are rare.

### PSOROSIS B

Psorosis B, which is caused by the virus *Citrivir psorosis* var. *anulatum*[7] (7, 8), differs from psorosis A mainly in the production of symptoms on mature leaves and on small twigs and fruit, together with the greater extent and more rapid development of the bark lesions. The young-leaf symptoms are perhaps less common, but they are similar to those of psorosis A. Although psorosis B is more deleterious to affected trees than psorosis A, the number of cases is comparatively few.

#### Bark Symptoms

Bark scaling on the trunk and larger limbs, with psorosis B, is similar to that of psorosis A, but gumming is more profuse in advance of scaling and less profuse after scaling occurs. Psorosis B also advances more rapidly than is usual with psorosis A, generally along one side of a trunk or branch (Fig. 6, A and B) and even into much smaller, more recently formed limbs and twigs. Elongated strips of bark may be killed through to the wood on trunk or limbs, and twigs are killed rapidly. Frequently, the outer bark is killed so rapidly that larger, longer flakes or scales are formed than at other times. Such large scales, however, may occur also with psorosis A.

Gumming, splitting of bark, and scaling of twigs of all sizes throughout the tree are common accompaniments of psorosis B and help to distinguish it from psorosis A, which rarely shows lesions on very small branches or twigs.

On twigs or water sprouts growing on badly diseased trunks, spots resembling those on mature leaves, with or without rings, occur on the green bark. As the water sprout matures, the raised portions of the spots become corky, glazed, and hard, and some of the spots resemble those commonly produced in leprosis (5, 13).

#### Wood Symptoms

Wood symptoms, both primary and secondary, are also found in psorosis B (Fig. 6, C), but the time between the formation of the primary and secondary lesions in the wood may be much shorter than in psorosis A. No distinct differences in wood lesions of psorosis A and psorosis B have been observed.

#### Leaf Symptoms

On the young, tender, rapidly growing leaves, up to the time they approach the hardening stage, the symptoms are usually similar to those of

[7] *Anulatum* = with a ring. This variety so named because of the ring spots which form on the mature leaves and on the fruit.

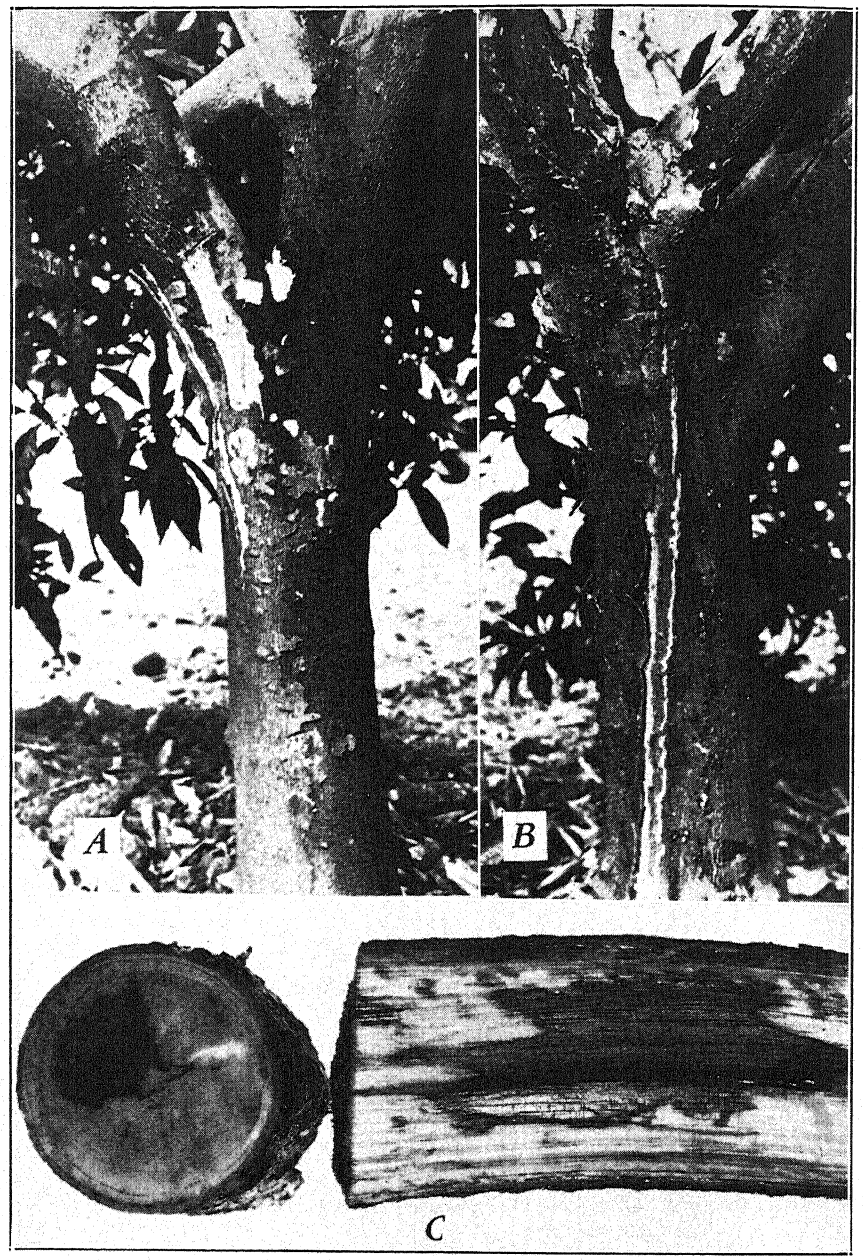



FIG. 6. Psorosis B on orange. A. Bark lesions on Valencia-orange trunk and main branch, showing extensive scaling. × about ⅛. B. Bark lesions on navel-orange trunk, showing scaling and one strip killed to the wood. × about ⅛. C. Transverse and longitudinal radial sections, showing primary lesions as layers of gum near the periphery of the wood, and a secondary lesion as a region of discoloration deeper in the wood. About natural size. A and B photographed by L. J. Klotz.

the other varieties of psorosis but less commonly manifested. The symptoms on the mature leaves, however, are, for the most part, different from

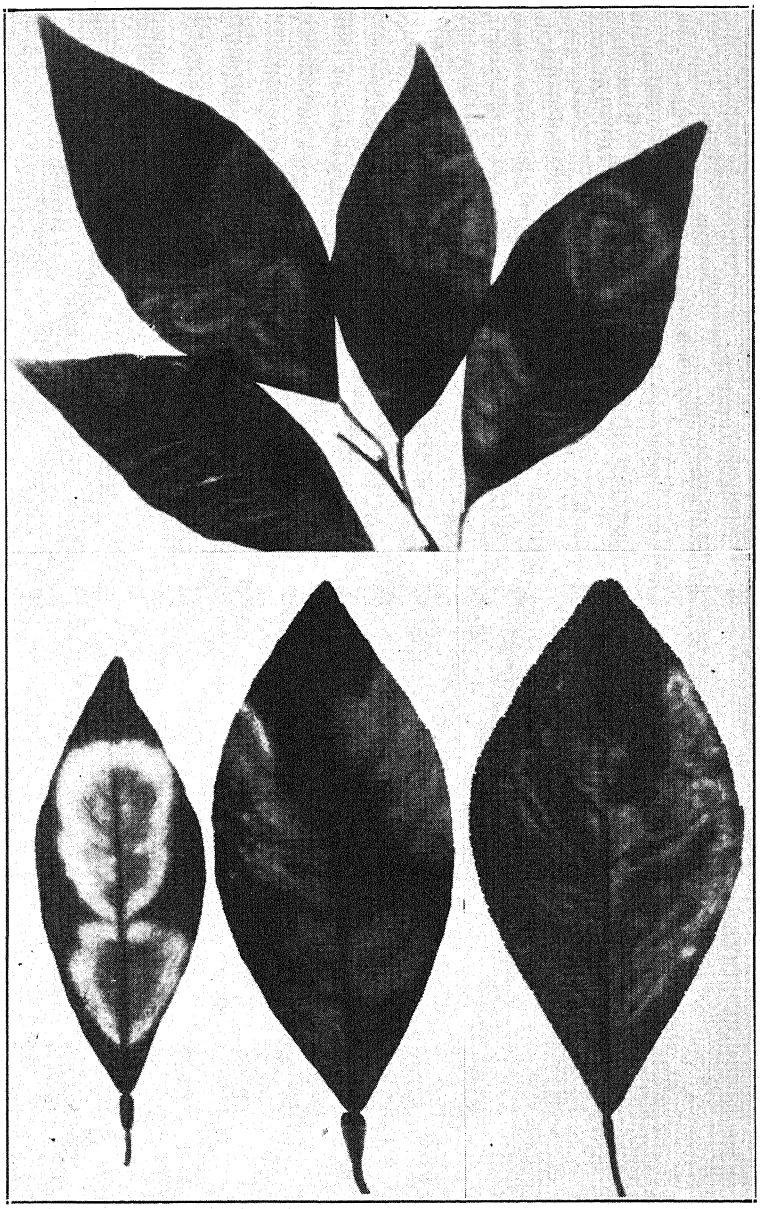


FIG. 7. Psorosis B spots on mature orange leaves, showing several patterns. × about ⅔. Top illustration photographed by L. J. Klotz.

those of any of the other psorosis varieties, except in certain severe cases of psorosis A, in which a few mature leaves may have spots resembling some of those that are common on psorosis-B trees.

The mature-leaf spots are of several kinds (Fig. 7). They range in size and appearance from mere dots to large, translucent areas, more or less circular and frequently in the form of rings. In the most conspicuous form, the spots may be large and may occur on any portion of the blade, often just above the petiole and across the entire blade. The central portion of such a spot is light to yellowish green, with or without a light-yellow circular-to-irregular border. Some spots may be in the form of single rings, concentric rings, or partial rings, occasionally with a tendency to necrosis in a portion of the rings. Another form of spot is made up of small, corky, raised areas forming a pustule or cluster of gum-filled dots surrounded by a halo and simulating certain types of leprosis (5, 13). Some of these raised dark areas are due to greater cell division in the tissue below, and to the deposition of certain products in some of the layers of cells near the surface.

In the greenish-yellow areas of mature leaves without pustules, the cells, under the microscope, appear normal in size, shape, and arrangement. In the pustular, corky areas, however, cork is produced by phellogen layers located in the palisade tissue of the upper leaf surface and in the spongy parenchyma of the lower leaf surface. The phellogen originates subjacent to cells with brown contents. Scattered groups of brown cells are sometimes seen in the mesophyll (15).

## Fruit Symptoms

In some fruits of trees infected by psorosis B (very rarely in fruits of trees infected by psorosis A), surface rings bordered by sunken grooves varying in size and pattern sometimes develop. On young green fruits, circular spots similar to those on mature leaves sometimes occur. These spots are light green or light yellowish. Sometimes most of the interior of the spot remains green and is surrounded with a yellow or pale-green band or ring. A band of narrow concentric rings often occurs, accompanied by necrosis, as described for mature leaves. In other cases, especially on grapefruit, large circular grooves, partial circles, or irregular curves where rings overlap, occur on the rind and often result in a rough, bumpy condition (Fig. 8).

### CONCAVE-GUM PSOROSIS

The most distinguishing features of concave-gum psorosis, which is caused by the virus *Citrivir psorosis* var. *concavum*[8] (8), are the concavities of various sizes that develop on the trunks and larger limbs. Young-leaf symptoms, similar to those of the other varieties of psorosis, are characteristic of the disease. During certain seasons, usually in the spring, the young-leaf symptoms of this variety tend to be of the zonate or oak-leaf-pattern type (Fig. 2). Although this pattern is not confined to leaves from concave-gum trees, it occurs there with sufficient regularity and uniformity during certain growth flushes to permit its use as one of the diagnostic characters. During the summer and fall, the oak-leaf pattern is usually absent.

[8] *Concavum* = concave.

## Bark Symptoms

For the most part, a fairly normal bark covers the surface of concavities (Fig. 9) of varying sizes and depths on the trunk or large branches. Several concavities near together often give the appearance of a flattening of the trunk or branch (Fig. 9, A). In the central part of the concavity, or around the outer rim, cracking of the bark often occurs, with gum oozing to the surface. A small amount of scaling, somewhat resembling that of psorosis A, is occasionally associated with the development of this variety of psorosis.

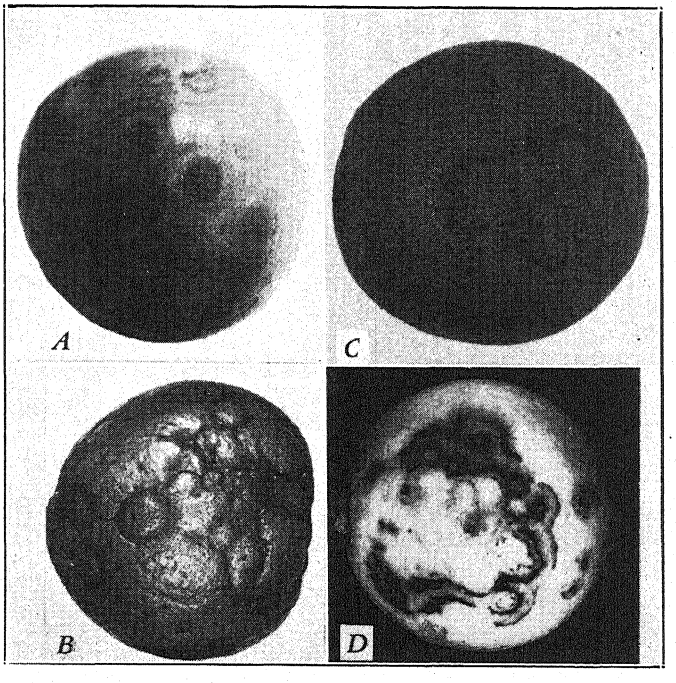



FIG. 8. Psorosis B on fruits. A and B. Depressed rings or grooves on grapefruit. C and D. Irregular grooves in the rind of oranges. × about ½. C and D photographed by L. J. Klotz.

## Wood Symptoms

With concave-gum psorosis, the primary symptoms in the wood usually are limited to gum development in a very definite local area just under the concavity; such gum development is rarely extensive. As long as the concavities remain few, no secondary lesions develop in the wood, and the tree appears not to be seriously handicapped in its normal development (5). When lesions become very numerous, dwarfing and declining may occur.

The gumming in the wood vessels in the early stage of this psorosis is confined to a very small region, only a few millimeters wide in transverse section and slightly more in radial section. This region enlarges gradually as the trunk or branch produces new layers of wood, so that in the subse-

quent consecutive layers, as seen in a cross section, there is a corresponding increase in width of the wood layer in which the formation of gum takes place (Fig. 10, A). Usually, however, the gum is confined to the periphery of the altered portion of the later wood layers. In the center of the cavity, there is little or no gum formation, but the wood tissues are visibly altered and considerably thinner than in the same layer of normal wood outside the lesion. This confinement of the gum to the increasing periphery of the altered portion in the successive wood layers gives the same kind of overlapping as that occurring in psorosis A and B, previously described (compare Figs. 4 and 6), but in a more restricted way. Owing to the successive, thinner layers of wood formed at the center of the lesion, a depression is

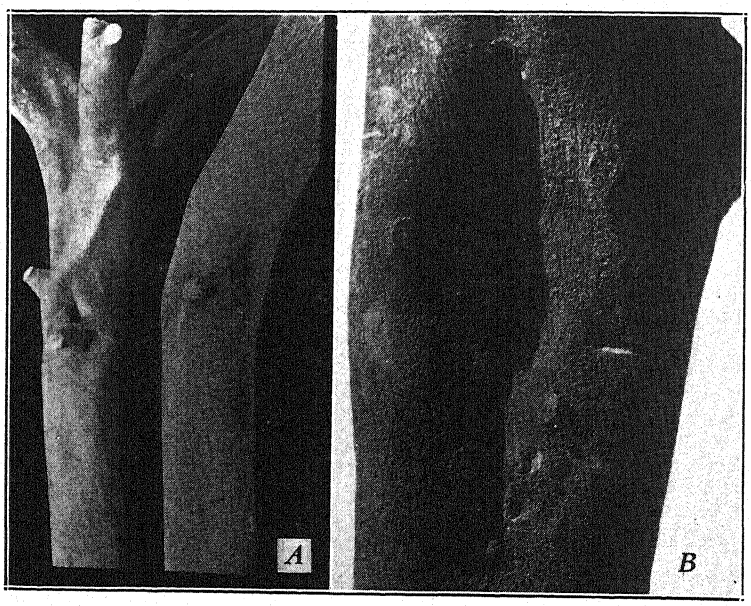



FIG. 9. Concave-gum psorosis. A. Concavities and suppressed growth in regions on orange branches. × about ½. B. Concavity on large branch, with a small amount of scaling. About natural size. Photographed by L. J. Klotz.

formed, which increases in size as the trunk or branch develops and finally produces the characteristic cup-shape concavity of concave-gum psorosis.

In the center and deepest portion of a concave-gum lesion, there frequently is found a more or less limited region where the tissues have, at some previous time, apparently been entirely inhibited, and where there is an abundance of gum. In some cases the trace of a dead twig can be detected at the same spot. In figure 10, B, which shows a cross section of a limb of sweet orange about 5 cm. in diameter, consecutive regions can be detected where extremely pronounced alterations of the wood took place during the early formation of the concave-gum lesion. The first pronounced alteration apparently took place when the limb was still quite young, and is restricted to a portion of the wood layer about 4 mm. wide in cross section. A callus

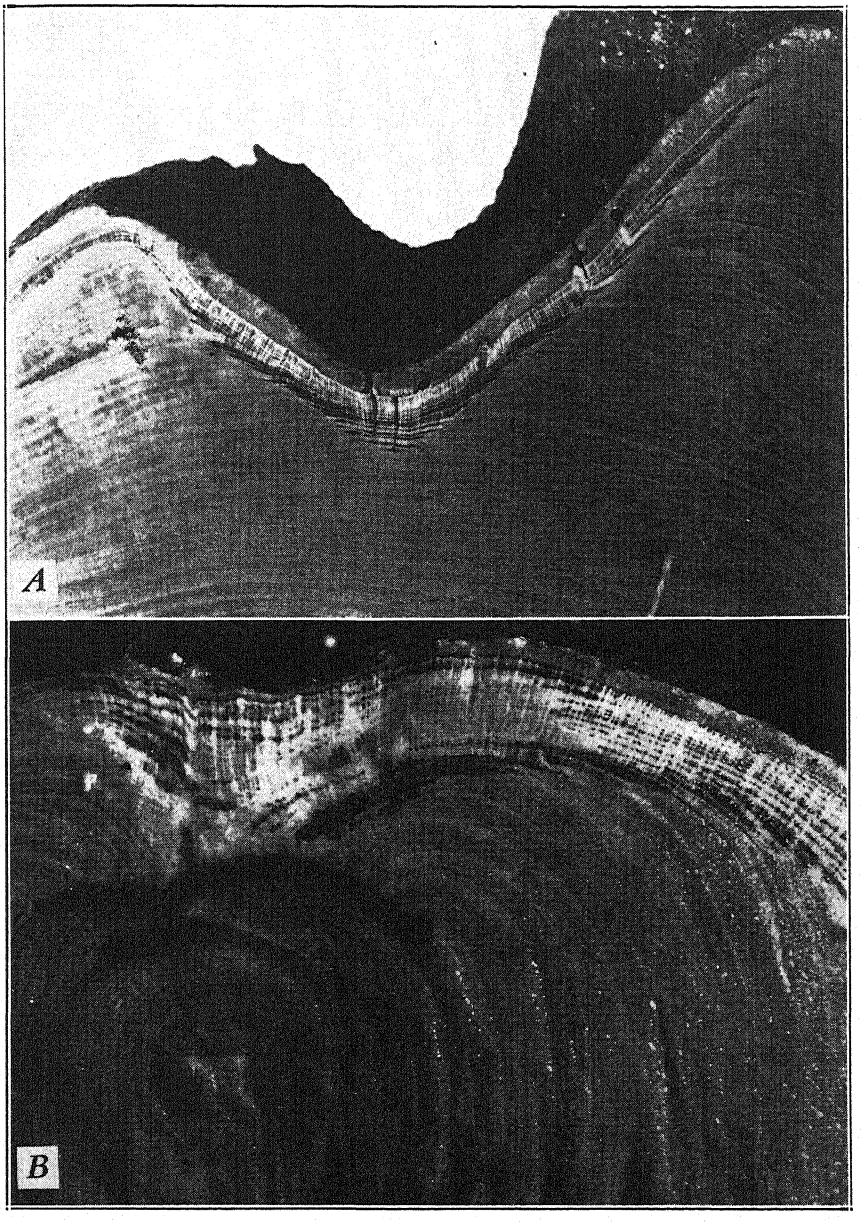



FIG. 10. Transverse sections through concave-gum concavities on sweet orange. A. Section from a lesion on the trunk, showing suppressed wood growth at the bottom of the concavity and overlapping layers of gum between layers of wood on the sides. $\times 1\frac{1}{2}$. B. Section from a lesion on a branch, showing two regions of severe injury to wood, followed by the usual development of overlapping gum layers between wood layers and a partial recovery at the bottom of the concavity. $\times 3\frac{3}{4}$.

formed and covered this portion, in which the cambium either had been killed or, for other reasons, could produce no wood. Later, a new region of dead tissue, 14 mm. in width, appeared, but this region was subsequently covered with wood. From then on, gum formation took place in overlapping layers, the amount of gum diminishing substantially as new layers were formed.

### BLIND-POCKET PSOROSIS

This variety of psorosis is caused by the virus *Citrivir psorosis* var. *alveatum*[9] (8). It appears on the trunk and large limbs of the tree in two forms: the most common form is a troughlike pocket in bark and wood; the other is eruptive, with bark scaling. The young-leaf symptoms are similar to those of the other psorosis varieties; no symptoms on mature leaves have thus far been observed.

#### Bark Symptoms

The bark in the noneruptive form of blind-pocket psorosis is usually normal in external appearance on the irregular surfaces of the narrow troughlike depressions. These lesions are often only 1 to 2 inches in length, but sometimes in older trees several lesions may run together to form much longer depressions or furrows (Fig. 11, A). The pockets usually differ from those of concave-gum psorosis in that they are narrower, more abruptly depressed troughlike depressions, frequently with two almost straight or even convex sides coming together at an acute angle at the bottom. In this angle at the bottom of the pocket or troughlike depression, the bark often is compressed or pinched by the growth of the wood on the two sides. The two sides sometimes grow together at the angle and leave a line of buried bark below. The bark often partially fills the depression and becomes thicker than the normal bark. Some cases of blind-pocket psorosis show longer or shorter depressions than others, with less abrupt slopes more nearly resembling those of the depressions of concave-gum psorosis, but distinguished from that variety by the difference in wood symptoms.

The eruptive blind pocket (Fig. 11, B) usually occurs within an area occupied by several blind pockets. It somewhat resembles bark lesions of psorosis A, for which it is easily mistaken. The scales of bark are much thicker and are usually larger than most of those of psorosis A. The possibility that such cases may be a mixture of blind-pocket psorosis and psorosis A is being investigated.

#### Wood Symptoms

Secondary wood symptoms consisting of internal wood discolorations are rarely produced by blind-pocket psorosis. The primary symptoms in the wood adjacent to the pockets consist in altered tissue varying in color often from yellow to ocherous-salmon. This tissue is composed of a rather loose wood parenchyma, often impregnated with either a waxy or a gummy sub-

[9] *Alveatum* = hollowed out like a trough.

stance. The apparent point of origin may be found 1 to 2 inches below the bottom of the pocket. Only rarely is gum exuded to the surface at the bottom of these pockets.

FIG. 11. Blind-pocket psorosis. A. Troughlike depressions or pockets on the trunk and main branches of an orange tree. B. Eruptive form of blind-pocket psorosis, showing thick scales of bark in region of pockets. Photographed by L. J. Klotz.

A cross section of a lesion shows that there is no considerable increase in the extent of the diseased portions of the successive wood layers; there is, however, a marked increase in the intensity of the effect. The affected por-

tion produces only an extremely thin layer of wood, as compared with the thickness of the same layer on either side of the affected portion (Fig. 12, A). In radial sections the lesion is seen to be elongated, often 3 to 8 cm. in length (Fig. 12, B). As an increasing number of wood layers are thus affected,

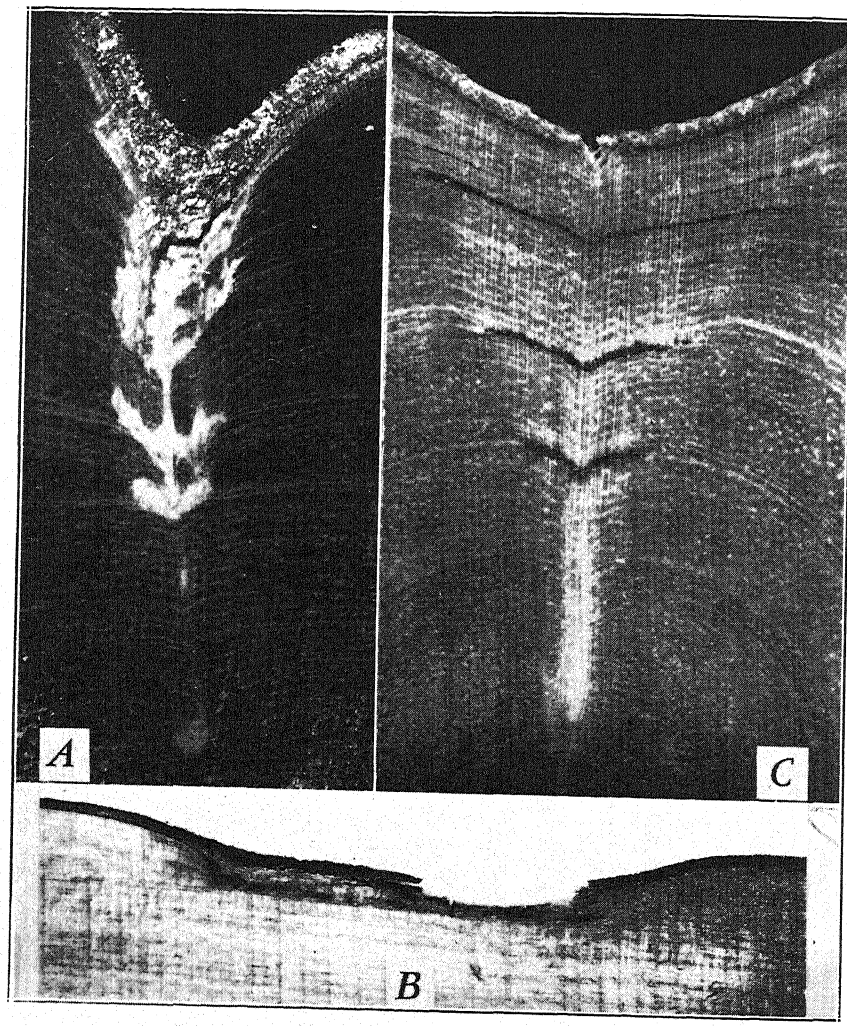



FIG. 12. A. Transverse section through a blind-pocket lesion, showing the deep origin of the pocket and the wood lesion consisting of loose parenchyma cells with local inhibition of normal wood. ×4½. B. Longitudinal radial section through one side of the pocket, the elongated region of wood inhibition about natural size. C. Transverse section through a concave-gum-like lesion. This lesion apparently began in a way typical of blind-pocket psorosis and later changed into a form characteristic of concave gum, with its overlapping layers of gum. ×4½.

a deep depression is produced, corresponding to the narrow, elongated region where the wood layers are extremely thin or are replaced by the loose wood parenchyma.

COMPARATIVE ASPECTS OF PRIMARY SYMPTOMS IN WOOD OF PSOROSIS A, PSOROSIS B, CONCAVE-GUM PSOROSIS, AND BLIND-POCKET PSOROSIS

A comparison of the primary lesions in the wood in cases of psorosis A, psorosis B, concave-gum psorosis, and blind-pocket psorosis, shows that, in a broad aspect, they differ chiefly in the extent of the alteration produced (12). In each of the 4 varieties of psorosis, the wood lesion apparently results from an alteration at or near the cambium in the affected region. For some reason not yet well understood, the cambium in that particular region ceases to produce normal wood.

With blind-pocket psorosis (Fig. 12, A and B), the alteration of the cambium is intense in its effects but limited in area. With psorosis A (Fig. 4), and generally with psorosis B (Fig. 6, C), the alteration of the cambium occupies an oval area, which progressively increases in size. The effect in the wood is a gummous degeneration of the vessels and other elements of the wood, leading to the formation of gum. With concave-gum psorosis (Fig. 10), the alteration in the wood near the cambium is intermediate between that of psorosis A or B and that of blind-pocket psorosis; although there is a progressive increase in the area affected, it is not so rapid nor so extensive as in psorosis A or B. The similarity in the nature of the overlapping layers of gum in psorosis A and in concave-gum psorosis is apparent when the figures are compared. The only real difference is the much greater extension of the layers with psorosis A or B.

This comparatively rapid extension of the layers of gum in psorosis A and B, closely following the extension of the bark lesion, eventually involves the whole, or a large area, of the circumference of the trunk, limb, or branch. When this occurs, there is a girdling effect, its severity perhaps being in proportion to the extension of the bark lesions, and to the completeness of the walls of gum within the outer wood layers.

The differences between concave-gum and blind-pocket psorosis might be considered greater than those indicated in the above comparison, because with the latter variety of psorosis, layers of gum usually are not formed in the wood. At times, however, it is difficult to distinguish between the lesions of concave-gum and blind-pocket psorosis, especially when conditions are more or less intermediate between the two extreme cases described. One such intermediate case is shown in figure 12, C; it was at first difficult to decide whether this was blind pocket or concave gum. The lesion apparently started in a way typical of blind pocket, but after a number of layers of wood had been formed without appreciable increase in the size of the affected portion, growth continued in a way usually observed in connection with concave gum.

Figure 12, C, shows that these gum layers are generally more pronounced in the portion of the wood layers that are formed early in the spring, where, in specimens of healthy wood, the vessels are more numerous and of greater lumen, and where the starch-filled wood parenchyma is more abundant.

The similarity in the primary lesions of the wood in psorosis A, psorosis B, concave-gum psorosis, and blind-pocket psorosis is a further evidence of the relationships between those diseases, already indicated by the similarity of their young-leaf symptoms.

### CRINKLY-LEAF PSOROSIS

Crinkly leaf (5, 13), seen principally on lemons, appears associated with psorosis in the following ways: If sweet orange is used as the rootstock, the orange stock of the affected tree, as it becomes old enough, will have bark symptoms, usually of psorosis-A type, while the young lemon leaves of the top, as well as the young orange leaves from rootstock suckers, will have both crinkly leaf and the young-leaf symptoms typical of the psorosis varieties. The lemon part of the tree does not develop bark lesions; neither does the lemon part develop wood lesions, unless these first originate in the sweet-orange stock and advance into contiguous lemon wood.

These crinkly-leaf and young-leaf symptoms have been transmitted by budding and bark grafting from lemon to lemon and from lemon to orange, in the same manner as has psorosis A. But, while strains of psorosis A are nearly always associated with crinkly leaf, a condition suggesting that crinkly leaf may be a symptom of psorosis A, not all strains of psorosis A are found to induce crinkly leaf on lemons.

There are at least 3 possibilities in the relationship of crinkly leaf to psorosis: (a) that it is a manifestation on lemon of the presence of a strain of psorosis A; (b) that it is caused by a mixture of the psorosis-A virus and some other virus variety; and (c) that it is due to a distinct variety of psorosis virus. In the present uncertainty as to relationship, no distinct virus name is employed.

In some cases there appears to be a greater tendency for large, longitudinal cracks, known as growth cracks, to form in the bark of affected lemon trees than in corresponding psorosis-free lemon trees, but the connection of such symptoms with psorosis is unconfirmed. The presence of shell bark (5) on lemon bark should not be interpreted as showing the presence of psorosis virus. Shell bark somewhat resembles bark symptoms of psorosis. The former is caused by the fungus *Phomopsis citri* and should not, therefore, be mistaken for a symptom of psorosis. So far as is known, crinkly leaf is not accompanied by any distinctive bark or wood symptom.

## Leaf and Fruit Symptoms

The young-leaf symptoms or small cleared areas observed on immature leaves in connection with crinkly leaf on lemon, are similar to the young-leaf symptoms of psorosis on orange. The mature leaves, and sometimes the young leaves, show a warping and pocketing that appear to be due to irregularities in growth in different areas or parts of the leaf blade (Fig. 13). Thickening and dwarfing of the leaves occur frequently, but not always.

The leaf effects caused by the bud mite, or other insects, resemble but should not be mistaken for true crinkly leaf.

The expression of the crinkly-leaf symptom varies on different branches and in different growth cycles. Sometimes the leaves of one cycle show severe pocketing and crinkling, while those of the next show this but slightly.

FIG. 13. Crinkly-leaf psorosis on lemon. Leaves with variegated light areas and crinkled, warped blades; lemon fruits coarse and rough. Note flecks in young leaves (upper left) and bumps on young fruit (upper middle). × about ¾. Photographed by L. J. Klotz.

Certain branches or leaves may show it severely; others, only slightly or not at all. Orange leaves are usually resistant to the crinkly-leaf effect, although they frequently show a slight crinkling.

The fruits on lemon trees affected with crinkly leaf usually tend to be coarse and rough, the fruit surface, in severe cases, being covered with irregular bumps (Fig. 13). Only occasionally have ring spots been found associated with crinkly leaf.

### INFECTIOUS VARIEGATION, ASSOCIATED WITH CRINKLY LEAF

Infectious variegation (11) is characterized by large, irregular chlorotic areas, white to yellowish white, which give the leaf blade a variegated appearance (13). This disorder has been transmitted along with the crinkly-leaf condition from lemon to lemon, from lemon to sour orange, and from lemon to sweet orange. All cases of infectious variegation seen so far on lemons in California, have been associated directly with crinkly leaf; it is possibly a phase of the latter. Striking examples of infectious variegation, from a lemon tree having crinkly leaf, are shown in figure 13. Several other crinkly-leaf strains have occasionally developed symptoms typical of infectious variegation along with the crinkly-leaf symptoms. Shoots with variegated leaves alternate with shoots showing no variegation. There is not sufficient evidence to separate, definitely, infectious variegation and crinkly leaf; the former will, therefore, be considered, tentatively, an occasional symptom associated with a crinkly-leaf complex.

### OTHER DISORDERS POSSIBLY DUE TO VIRUSES

Several abnormalities on citrus, whose transmission has not yet been satisfactorily proved, but are suspected to be due to viruses, are described. It should not be inferred that, because they are included in the present paper on psorosis varieties, they have been proved to be a part of the psorosis group. It is possible, however, that further study may show some of them to belong to this group.

#### Corky Bark

*Corky bark,*[10] so termed because of the development of corklike eruptions in the bark, takes on various forms, which are accompanied by varied effects on the tree itself. Gumlike substances are usually mixed with the corklike cells of these lesions, and the wood underneath may also be affected.

Leaf symptoms similar to those due to psorosis have been observed on 3 of the following forms of corky bark, as indicated; but the observed trees of the other 2 forms were not in a suitable condition of growth to determine leaf symptoms. It has not yet been determined whether or not the leaf symptoms observed are connected with the corky-bark disorders.

*Necrotic-cavity Corky Bark.* This type of corky bark, found by J. C. Perry on Valencia-orange trees, resembles psorosis in some respects. The lesions are in the form of necrotic cavities, the margins of which are comprised of corky, raised tissues impregnated with hard, vitreous, gumlike material, and small scales or flakes of bark. The bark in the bottom of cavities is dead and may crack off in flakes.

*Crumbly-gum Corky Bark.* Corky bark of this type occurs on orange trees in irregular, eruptive, gall-like formations and ridges, in most of which gum and broken tissue together form light-brown crumbly masses extending out from the surface (Fig. 14, A). In larger, older lesions, the wood tissues

[10] The term *corky bark* was first used by J. C. Perry of the East Highland Orange Company, for what is, in the present paper, called *necrotic-cavity corky bark.*

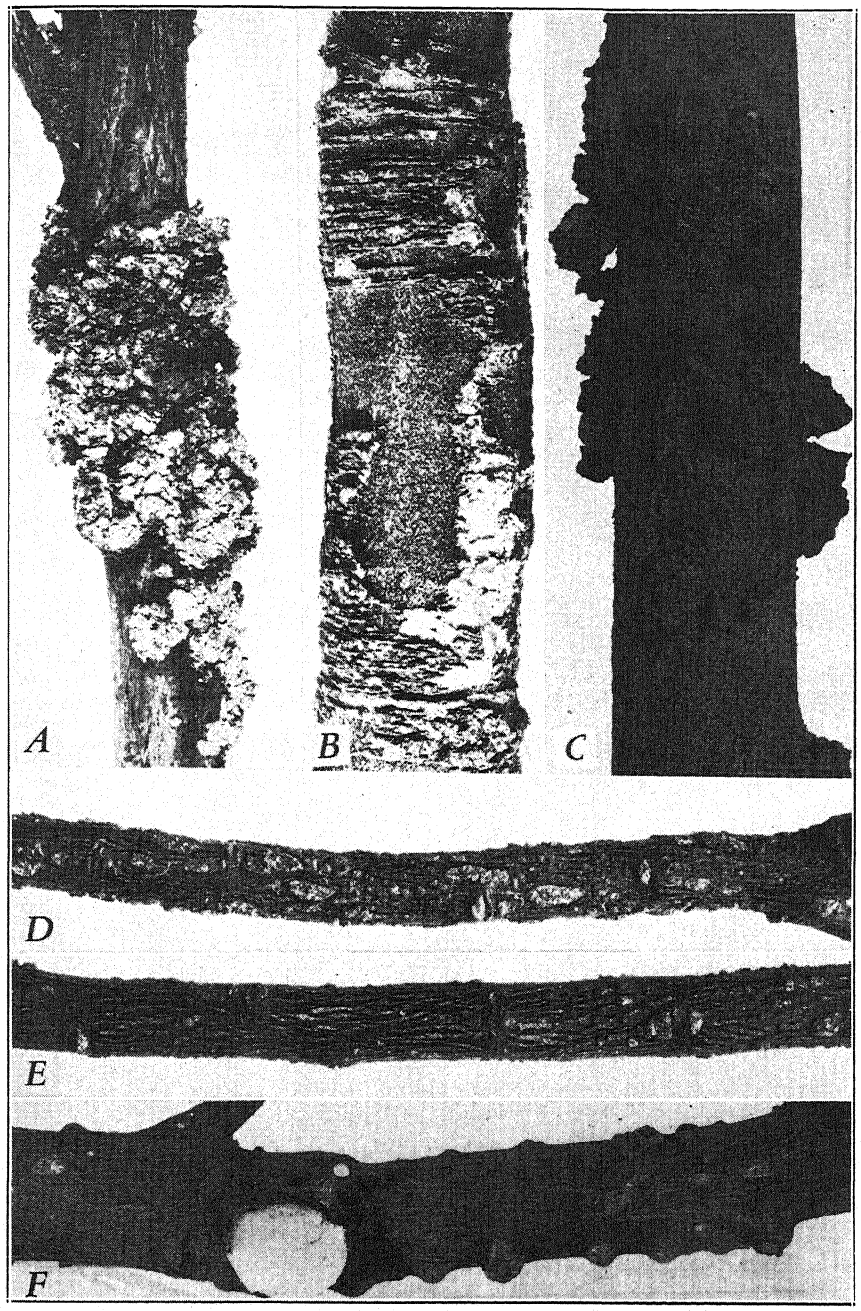



FIG. 14. Corky bark and knobby bark. A. Crumbly-gum corky bark, with erupted bark and brown gum intermingled. B. Banded corky bark on Valencia orange, showing raised horizontal bands of corklike eruptions. C. Circular-spot corky bark on navel orange, showing circular regions of erupted, corklike layers of bark. D and E. Tattoo-netted corky bark on Valencia orange, showing the irregular areas of erupted bark often suggesting a kind of tattoo. F. Knobby bark on branch of navel orange, each knoblike projection consisting of a gnarled, spherical wood interior covered with slightly altered bark. A to E, × about $\frac{3}{4}$; F, × about $\frac{1}{3}$. D and E photographed by L. J. Klotz.

contain dead portions and grow in an irregular, twisted, gnarled manner with much mixing of bark and wood in the center. Gum forms in layers in the wood as in psorosis. In some gall-like lesions, 10 to 12 mm. in diameter, abnormal wood grows into budlike projections, greenish at the periphery but necrotic within. Young-leaf symptoms similar to those of psorosis have been found on affected trees.

*Banded Corky Bark.* This form of corky bark, found by I. B. Suryieh on Valencia orange, appears as raised bands of corky eruptions extending horizontally around, or part way around, the trunk or limb of the tree. These may occur as narrow horizontal bands or ridges, 1 or 2 mm. wide and the same distance apart, over an area several inches long, as shown in figure 14, B. Layers of gum alternating with layers of wood occur directly inward from the bark lesion and extend somewhat beyond the margins of the lesion.

*Circular-spot Corky Bark.* The circular regions of erupted, corklike layers of bark formed in this type of corky bark are shown in figure 14, C. Circular-spot corky bark has been found on navel-orange trees and bears a remote resemblance to some types of psorosis A.

Young-leaf symptoms similar to those of psorosis A have been found and have been transmitted. More time is necessary to determine whether the bark symptom is also transmitted. At the periphery of the lesions, segments of corklike projections, some of which are 4 to 5 mm. in height, are formed. Each segment shows closely set layers. Sometimes the oldest part of the segment is turned back against the bark. The middle of the lesions also remains rough, the older projections having sloughed off. Other cases of circular-spot corky bark have concentric ridges of slightly raised corky tissue. Wood lesions are present beneath the bark lesions and are not unlike the primary lesions of psorosis A, with overlapping layers of gum.

*Tattoo-netted Corky Bark.* The irregular, elongated areas, spots, and ridges of corky bark sometimes found on trunk and limbs of Valencia-orange trees, often forming patterns (Fig. 14, D and E), suggested the name *tattoo-netted.*[11] Branch growth of affected trees often shows curved twigs and shortened nodes. Young-leaf symptoms similar to those of psorosis occur on some trees and have been transmitted by tissue fusion. Badly diseased trees often have eruptive areas similar to those of psorosis A, with primary and secondary lesions in the wood or areas resembling eruptive blind pocket.

## Knobby Bark

Knobby bark[12] is a condition distinguished by the presence of knobs or hard, gall-like projections covered with nearly normal, unbroken bark. These knobs are of various sizes, ranging from 1 to 2 inches across and projecting $\frac{1}{8}$ to $\frac{1}{2}$ inch or more from the usual contour of the bark. An example of occurrence was that on a navel-orange tree, on branches 1 to 3 inches in diameter (Fig. 14, F). The bark over these knobs is about the same thick-

[11] The name first suggested for this disorder by I. B. Suryieh.
[12] Found and named by J. C. Perry of the East Highland Orange Company.

ness as in other areas. The hard, firm wood tissue underneath the knobs is more easily cut than normal wood, is light-brownish, and occurs as a spheroid core extending down into, or embedded in, the normal wood. It is separated from the normal wood by a definite margin and leaves a crater-like opening when cut out. This abnormal spheroid region of wood tissue, the knob, shows growth in circles and curves, with a light, gumlike substance within. No young-leaf symptoms were seen on trees with knobby bark.

## SUMMARY

The term *psorosis* is expanded to include a group of similar disorders on citrus, previously considered to be the effects of different diseases, but now thought to be due to varieties of one virus, *Citrivir psorosis*.

The varieties of psorosis, accordingly, now include psorosis A, psorosis B, concave-gum psorosis, blind-pocket psorosis, and crinkly-leaf psorosis.

All these varieties of psorosis have, in common, a mosaiclike symptom in young leaves, characterized by white to yellowish flecks in the region of the veinlets or cleared bands along the veins and veinlets. These varieties also have fundamentally similar alterations in the wood producing the primary symptoms.

Psorosis A and B, caused by *Citrivir psorosis* var. *vulgare* and *C. psorosis* var. *anulatum,* respectively, show the following symptoms in addition to young-leaf symptoms: (a) bark lesions, characterized by a scaling of the outer bark in dry, irregular flakes, or by erumpent pustules; and (b) wood lesions of two kinds: primary lesions near the cambium, consisting of layers of gum between layers of wood directly under the bark lesions; and secondary lesions consisting of discolored wood, usually farther inward. The regions of the secondary lesions and contiguous regions of nondiscolored wood are devoid of starch and are impermeable to the passage of water.

Psorosis B differs from psorosis A in having more rapidly developing and more continuous areas of bark scaling, in having numerous twig lesions, and in having, on mature leaves and on fruit, ringed spots, which rarely occur with psorosis A.

Concave-gum psorosis, caused by *Citrivir psorosis* var. *concavum,* is characterized by the development of concavities due to the inhibition, or slowing down, of wood growth in localized regions of the trunk or large branches. Gum layers formed in the wood in this type of psorosis are similar to those of psorosis A and B but more localized.

Blind-pocket psorosis, caused by *Citrivir psorosis* var. *alveatum,* usually appears as troughlike depressions in trunk or limbs, due to inhibition of wood growth in even more localized and restricted regions than in concave-gum psorosis. The sides of the depressions may be straight or convex. Underneath the lesion, the loose wood parenchyma is usually impregnated with either a waxy or a gummy substance.

The primary lesions in the wood of the 4 preceding varieties, considered

in broad aspect, have similar features and differ chiefly in the extent of alterations of the wood. In blind pocket, the alteration is intense but narrowly localized; in concave gum, less intense but somewhat spread; while in psorosis A and B, the alteration is spread out over large regions and may have even a girdling effect on trunk and limbs.

Crinkly-leaf psorosis is evident mainly on lemons and develops, in addition to the young-leaf symptoms, a warping and pocketing of mature leaves. The fruit is usually rough and coarse with irregular bumps. There are no distinctive bark or wood symptoms.

Infectious variegation, which is characterized by irregular chlorotic areas on the leaf blade, appears occasionally on lemon leaves. Since there is not sufficient evidence to separate infectious variegation and crinkly leaf, the former is tentatively considered an occasional symptom associated with a crinkly-leaf complex; both, when they occur, are usually found associated with psorosis A.

Other possibly virus effects, which may be related to the preceding psorosis varieties, are corky bark and knobby bark. Five kinds of corky bark have been observed: (a) necrotic-cavity, (b) crumbly-gum, (c) banded, (d) circular-spot, and (e) tattoo-netted.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA

## LITERATURE CITED

1. BARTHOLOMEW, E. T. Internal decline (endoxerosis) of lemons VI. Gum formation in the lemon fruit and its twig. Amer. Jour. Bot. 15: 548–563. 1928.
2. BITANCOURT, A. A., H. S. FAWCETT, and J. M. WALLACE. The relation of wood alterations in psorosis of citrus to tree deterioration. Phytopath. 33: 865–883. 1943.
3. FAWCETT, H. S. New symptoms of psorosis, indicating a virus disease of citrus. (Abstract) Phytopath. 23: 930. 1933.
4. ———. Is psorosis of citrus a virus disease? Phytopath. 24: 659–668. 1934.
5. ———. Citrus diseases and their control. 656 p. 2d ed. McGraw-Hill Book Company, Inc. (New York and London). 1936.
6. ———. Psorosis in relation to other virus-like effects on citrus. (Abstract) Phytopath. 29: 6. 1939.
7. ———. Suggestions on plant virus nomenclature as exemplified by names for citrus viruses. Science 92: 559–561. 1940.
8. ———. Citrus viruses. Phytopath. 31: 356–357. 1941.
9. ———, and L. C. COCHRAN. Symptom expression in psorosis of citrus as related to kind of inoculum. (Abstract) Phytopath. 32: 22. 1942.
10. ———, and L. J. KLOTZ. Types and symptoms of psorosis and psorosis-like diseases of citrus. (Abstract) Phytopath. 28: 670. 1938.
11. ———, and ———. Infectious variegation of citrus. Phytopath. 29: 911–912. 1939.
12. ———, A. A. BITANCOURT, and J. M. WALLACE. Wood alterations in psorosis of citrus in relation to tree decline. (Abstract) Phytopath. 32: 828. 1942.
13. KLOTZ, L. J., and H. S. FAWCETT. Color handbook of citrus diseases. 90 p. 40 plates in color. University of California Press (Berkeley and Los Angeles). 1941.
14. SWINGLE, WALTER T., and HERBERT J. WEBBER. The principal diseases of citrus fruits in Florida. U. S. Dept. Agr. Div. Veg. Physiol. and Path. Bull. 8: 1–42. 1896.
15. WEBBER, IRMA E., and H. S. FAWCETT. Comparative histology of healthy and psorosis-affected tissues of *Citrus sinensis*. Hilgardia 9: 71–109. 1935.

# THE RELATIONS OF WOOD ALTERATIONS IN PSOROSIS OF CITRUS TO TREE DETERIORATION[1]

A. A. Bitancourt,[2] H. S. Fawcett,[3] and J. M. Wallace[4]

(Accepted for publication March 3, 1943)

## INTRODUCTION

In a description of the symptoms of the different varieties of the psorosis of citrus, Fawcett and Bitancourt (6) point out that in psorosis A and psorosis B, caused by the 2 virus varieties *Citrivir psorosis* var. *vulgare* and *C. psorosis* var. *anulatum,* respectively (4, 5), there are, in addition to bark lesions, 2 types of wood lesions, primary and secondary.

The primary lesion forms in the wood soon after and almost coextensively with the lesion in the bark, and is characterized by gum layers between layers of either normal or altered wood. The secondary lesion appears to begin in older, more interior wood only after a considerable period of time, usually several years after the inception of the bark lesion and the primary wood lesion. The secondary lesion is marked by an irregular discoloration of the inner wood (3), with wood vessels partially or entirely filled with gum, the amount depending on the stage of development of the lesion, and by an absence of starch in both the discolored wood and a contiguous border surrounding the discolored area. The starch-free border follows almost exactly the pattern shown by the outer margin of the discolored part of the wood and also shows gum-plugged vessels. However, most wood vessels in the tissues contiguous to the discolored wood show accumulations of small quantities of gum on one or both sides of the perforation plates, as described earlier by Webber and Fawcett (8). This type of plugging is shown in figure 1, B and D. The gum plugs seem to be contemporaneous with the disappearance of starch and probably are derived from it (2). This secondary lesion of the more interior wood may finally extend until it includes the primary lesion near the bark. The degree of involvement of all the wood appears, in general, to be correlated with the degree of deterioration of the tree or part affected.

These wood alterations are accompanied by retarded growth and progressive deterioration of the branches and foliage. The growth of new twigs is less vigorous, the foliage becomes gradually less dense, the leaves become smaller and fall prematurely, and the twigs usually die back after many

[1] Paper No. 491, University of California Citrus Experiment Station, Riverside, California. Presented at the Pacific Division of The American Phytopathological Society, Salt Lake City, Utah, June, 1942. The joint work reported in this paper was done during the tenure, by the senior author, of a John Simon Guggenheim Memorial Foundation Fellowship for research at the University of California Citrus Experiment Station, Riverside, 1942.

[2] Plant Pathologist, Instituto Biologico, São Paulo, Brazil.

[3] Professor of Plant Pathology and Plant Pathologist in the Experiment Station, University of California.

[4] Associate Plant Pathologist in the Experiment Station, University of California.

leaves have been shed. Occasionally, individual branches die rapidly, and the leaves dry before they are shed. Even when the branch or trunk dies back to the discolored region, the roots remain alive and tend to send up new shoots.

Observation of many citrus trees has shown that the psorosis bark lesions and the primary lesions in the wood may be present for a considerable time

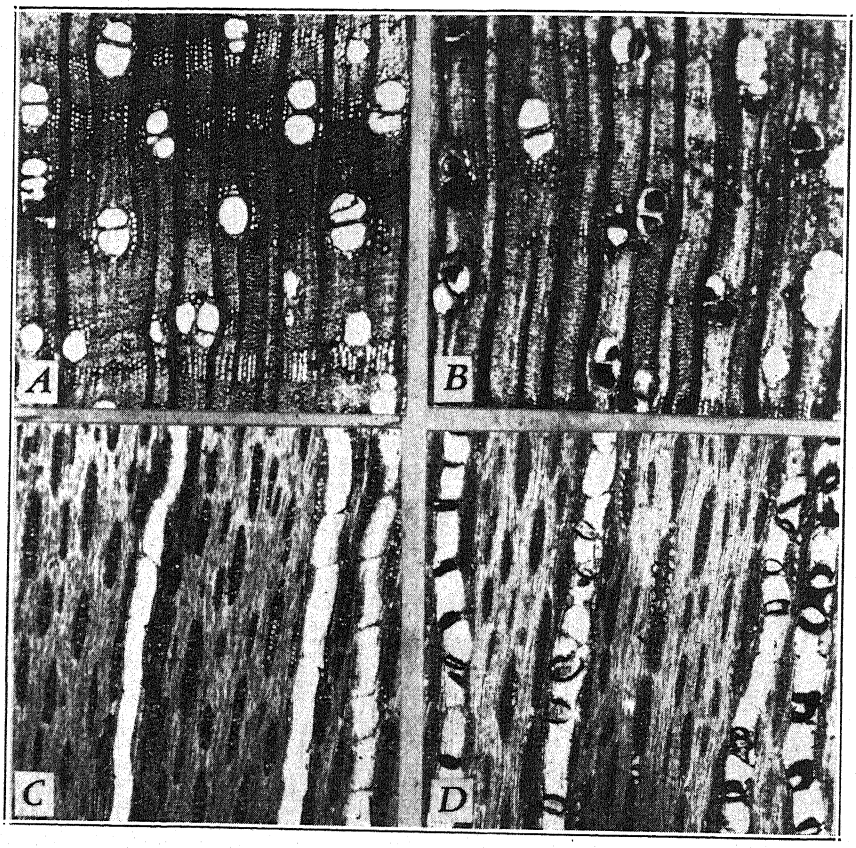



FIG. 1. A. Transverse section of wood of healthy orange tree. ×40. B. Transverse section of wood of psorosis-affected orange tree, showing gum plugs in xylem vessels in tissues contiguous to secondary wood lesion. ×40. C. Tangential section of wood of healthy orange tree. ×40. D. Tangential section of wood of psorosis-affected orange tree, showing accumulation of gum at perforation plates of xylem vessels. Sectioned from wood free of discoloration but near secondary lesion. ×40.

(several years in most cases) before any marked deterioration in growth or foliage takes place. It is only after the secondary wood lesions appear, with the visible discoloration of the wood in the interior of the trunk or branches, that noticeable deterioration takes place.

This paper presents the results of a study of wood alterations in relation to deterioration of psorosis-affected citrus trees.

### WATER-CONDUCTION EXPERIMENTS

The investigations of Webber and Fawcett (8) and of Fawcett and Bitancourt (6) disclosed that the xylem vessels of tissues in the vicinity of psorosis lesions were commonly filled or partly plugged with gum. This suggested that the deterioration of affected trees might be due to an insufficient conduction of water through the affected parts to the upper portions of the trees. Experiments were undertaken to determine the effect of primary and secondary lesions on water conduction through citrus wood.

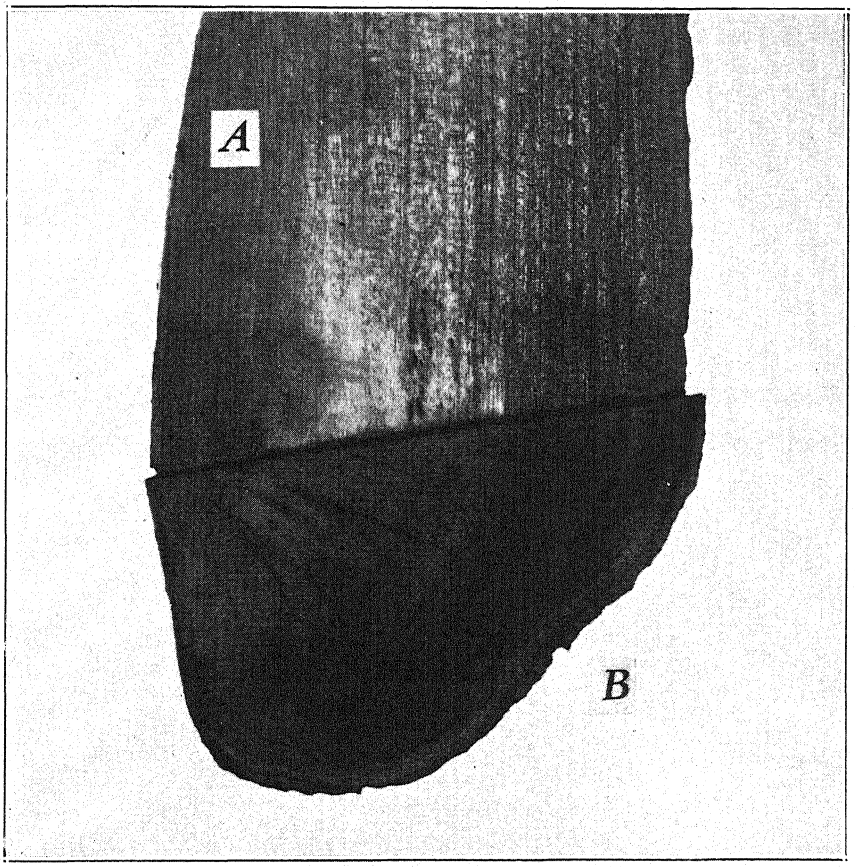



FIG. 2. Longitudinal (A) and transverse (B) sections of a block of orange trunk wood having a secondary lesion in the wood. The lower portion of the block had been dipped in an 0.5 per cent aqueous solution of safranin for a period of 7 hours. Later, the whole block was treated with the I–KI test for starch. Safranin stain, which penetrated the starch-containing regions of the healthy wood, is seen as the darkest area in the lower right portion of the longitudinal section (A) and as the darkest area in the transverse section (B). The irregular, starch-free, discolored areas of the secondary lesion, at the left, are shown surrounded by the lighter, starch-free portions of the lesion. Natural size.

### Preliminary Experiments

In preliminary experiments, the regions of the wood where the passage of water is likely to take place in diseased specimens, were determined by

the staining of wood elements with 0.5 per cent aqueous solutions of acid fuchsin or safranin.

Figure 2 shows longitudinal and transverse sections of a block of trunk wood, the lower end of which was maintained in a solution of safranin for over 7 hours. The solution was absorbed into the wood by capillarity and penetrated the wood vessels approximately $1\frac{1}{2}$ cm. In some vessels, however, the stain penetrated up to 4 cm. When the I–KI (iodine-potassium iodide) test for starch was applied to the same section, it was seen that the

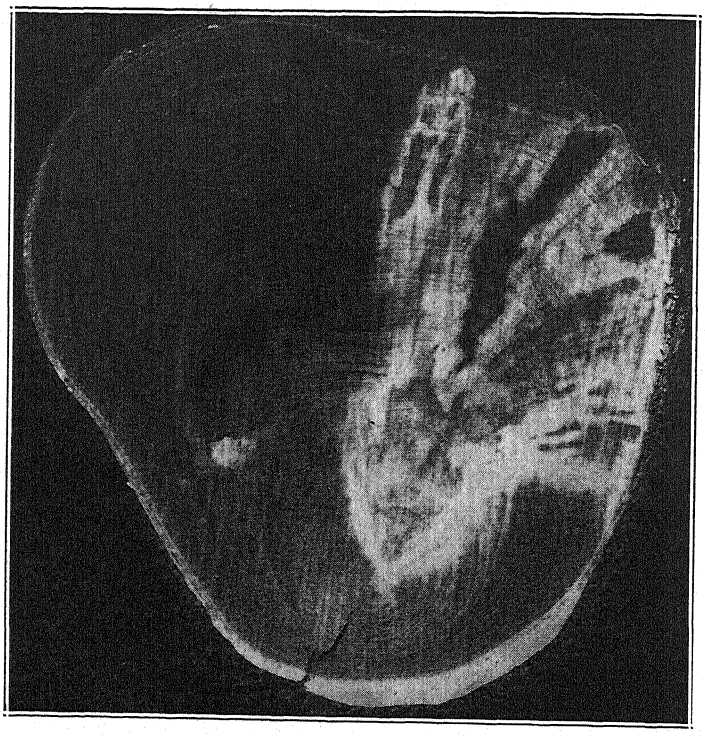


FIG. 3. Tansverse section of an orange branch, showing secondary lesions. The continuous dark portion (at the left and bottom) shows the presence of starch in the unaffected wood, as determined by the I–KI test. The irregular dark patches surrounded by white constitute the regions of the secondary lesions. Starch is absent or slight in these regions, and the passage of water is blocked. About natural size.

regions that the safranin solution had failed to penetrate were those showing little or no starch present. These were the regions of the visibly affected secondary lesions and the starch-free regions around them. This indicated that water passage was being interfered with in the discolored portion of the wood of the secondary lesion and also in a contiguous region, which could be revealed only by the absence of reaction in the I–KI test for starch. For example, in a section such as that shown in figure 3, treated with I–KI, water can pass in the black, starch-bearing portions of normal-appearing wood at the left and at the bottom, but is blocked in the remaining nearly starchless

portions of the secondary lesions, which show as white areas intermixed with irregular dark patches.

In other experiments, a solution of acid fuchsin was drawn into the cut branch or wood block by dipping one end into the solution while maintaining a partial vacuum at the other end.[5] In most experiments, sections of branches, or wood blocks, 5 cm. long were used.

These tests, like those of Villiers (7), indicated that in normal, healthy wood there was very little lateral movement of the stain. Most of the passage of aqueous stain took place in the peripheral region of the wood. The smaller, central portion allowed less stain to pass. The visibly affected wood allowed no stain to pass, and the adjacent wood usually acted in the same manner. For example, figure 4, A, shows the movement of the acid fuchsin stain in a block 7½ cm. in diameter and 5 cm. high, taken within close proximity to a discolored region of a secondary wood lesion. The effect of the I–KI test for starch is shown in figure 4, B, a section cut from wood contiguous to that shown in figure 4, A. The region of passage of acid fuchsin solution is seen to be identical with that of the positive starch reaction. This experiment also showed that passage of water was blocked in the discolored wood of the secondary lesion and in the contiguous starch-free wood. It may be concluded that the conducting vessels in these portions had been clogged.

TABLE 1.—*Time required for passage of the first 3 cc. (of 8 cc.) of water through each of three portions of a healthy block of wood 5 cm. in length, in five successive runs, under suction equivalent to four-fifths of an atmosphere*

| Location in block | Successive runs | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| | *Seconds* | *Seconds* | *Seconds* | *Seconds* | *Seconds* |
| a ..... | 42 | 48 | 56 | 75 | 103 |
| b ..... | 26 | 30[a] | 35 | 55 | 75 |
| c ..... | 53 | 58 | 70 | 107 | 130 |

[a] Interpolated.

In order to obtain a measure of the extent to which the wood alterations interfere with the passage of water, experiments were made in which a given number of cubic centimeters of water were drawn through specific portions of 5-cm. sections of branches or blocks of trunks and large limbs of affected trees. One end of the block was placed in water; at the other end a suction equivalent to approximately four-fifths of an atmosphere was maintained.

It is known that the amount of water or other fluids that can be passed through wood by forcing the fluids under pressure, or by suction at one end, the other end being immersed in the fluid, is extremely variable (1). There

[5] In this and following experiments, one end of the cut branch or wood block was dipped in water or stain solution, the free surface of which was, of course, at atmospheric pressure. A suction corresponding to approximately 60 cm. of mercury was then applied to the other end. This suction produced a difference of approximately four-fifths of an atmosphere of pressure.

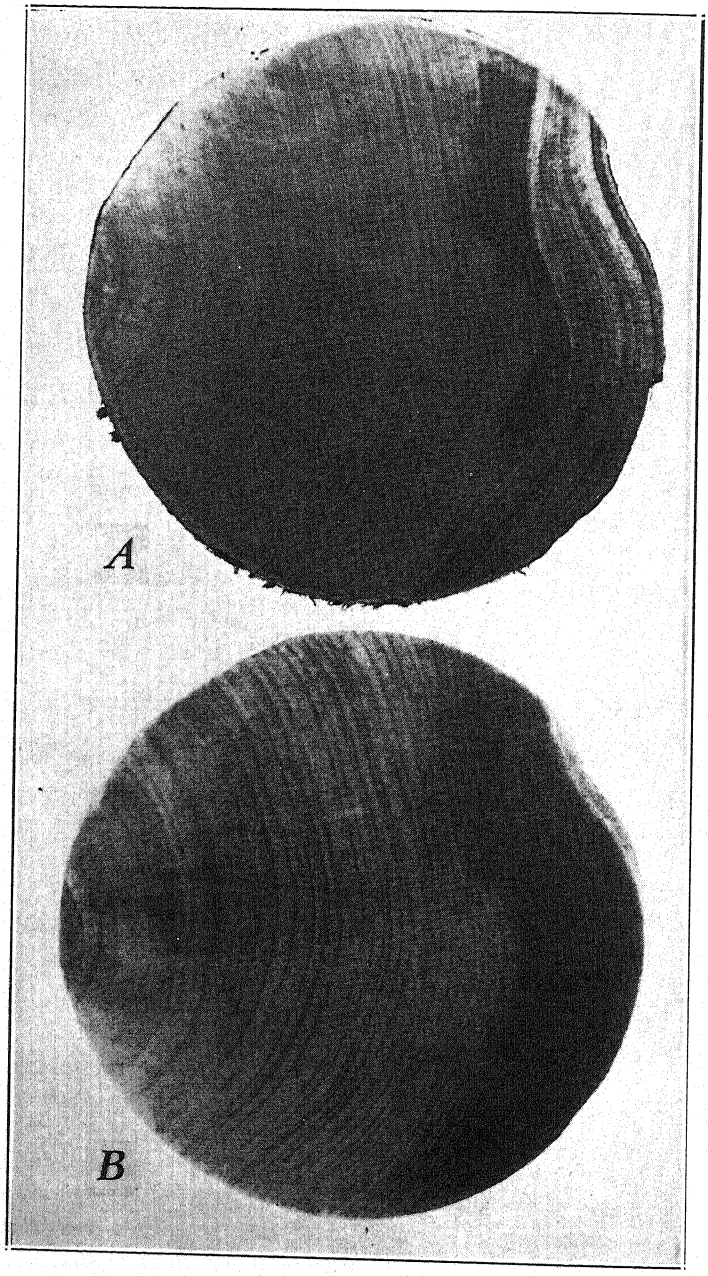



FIG. 4. A. Transverse section of a block of orange trunk wood taken slightly above a region showing the discoloration of a secondary lesion. The wood next to the bark shows staining resulting from an aqueous solution of acid fuchsin, which was drawn through the wood by suction. B. Transverse section of a block of trunk wood taken from the same tree, close to the preceding block. This wood was treated with I–KI and shows that the starch-containing region corresponds with that through which the fuchsin solution passed. The lighter, nearly starch-free region in which the passage of the fuchsin was blocked, as shown in A, was clogged with gum plugs as a result of its proximity to the secondary wood lesion. Natural size.

is a considerable decrease in the rate of flow with time, even during the short period involved in experiments in which only a few cubic centimeters of fluid are passed through the wood.

The results of one experiment with a healthy block of wood, $7\frac{1}{2}$ cm. in diameter and 5 cm. in length, are presented in table 1. In this experiment the time required for the first 3 cc. (of 8 cc.) of water to pass through each of 3 specific portions of the block, in 5 successive trials, was determined. The water was drawn through at 3 different places, each approximately 1 sq. cm. in area, on the surface of the block, near the bark. A difference of about four-fifths of an atmosphere was maintained in the pressure at the two ends of the block. It is apparent that the time required for water passage consistently increased with each successive trial, until in the fifth trial the time required was between two and three times that of the first trial. This table also shows that there is a considerable variation in the amount of water that can be drawn through different but similar portions of the same block of healthy wood, in a given period of time. There is also a distinct relationship between the amount of water passing in a given time and the width of wood layers: the wider the wood layers, the larger the amount of water passed. The difference between narrow and broad wood layers is shown in figure 6, A.

## Water Conduction in Wood Having Primary Lesions Only

Results of an experiment in which water was passed through two 5-cm. sections cut from a psorosis-infected branch 2.5 cm. in diameter, are shown in table 2. One of the sections had a bark lesion, with its accompanying

TABLE 2.—*The effect of bark and primary lesions on the rate of water passage, under suction equivalent to four-fifths of an atmosphere, through two sections of wood, each 5 cm. long and about 2.5 cm. in diameter, cut from the same branch of a psorosis-infected tree (experiment conducted as shown in Fig. 5)*

| Amount of water passed | Wood without lesions | | | Wood with lesions | | |
|---|---|---|---|---|---|---|
| | Successive runs | | | Successive runs | | |
| | 2 | 3 | 4 | 2 | 3 | 4 |
| *cc.* | *Seconds* | *Seconds* | *Seconds* | *Seconds* | *Seconds* | *Seconds* |
| 1 | 23 | 42 | 49 | 61 | 154 | 225 |
| 2 | 65 | 84 | 111 | 119 | 291 | 505 |
| 3 | 106 | 140 | 171 | 194 | 445 | 740 |

primary wood lesion; the other, cut beyond the lesions on the same branch, was lesion-free. Here again, the time required for the passage of equal amounts of water increased with each successive trial, the fourth trial requiring one and one-half to twice as much time as that of the second run in the lesion-free section. The fourth trial with the lesion-affected section required about 4 times as much time as the second trial with this same section. Although there was no evidence of a secondary lesion in the lesion-

affected block, the difference in the rate of water passage through the two blocks, throughout the experiment, was very marked. However, even though water was retarded by the bark lesion and primary wood lesion, the reduction in water passage was not sufficient to cause marked deterioration in such a branch.

This experiment shows that, if the amounts of water that can be passed through different specimens of citrus wood, or even different parts of the same specimen, are to be compared, not only should the same suction be applied, but other factors, such as the places where the suction is applied, the amount of water to be drawn, and the amount of water just previously drawn through, should be taken into consideration. In order to avoid the errors due to the extremely variable rate of flow resulting from these factors, most of the following experiments were made comparatively. Comparable sections of branches or blocks of wood, healthy and diseased, were collected on the same occasion and were cut and prepared in the same way. Water was drawn through these sections simultaneously, in the same apparatus, with the same differences in pressure.

TABLE 3.—*The effect of bark and primary lesions of psorosis on time required for passage of 10 cc. of water, under suction equivalent to four-fifths of an atmosphere, through sections of Valencia-orange wood, 5 cm. in length and 2 to 3 cm. in diameter*

| Date, 1942 | Experiment No.[a] | Wood without lesions | Wood with lesions |
|---|---|---|---|
| | | *Seconds* | *Seconds* |
| May 11 | 1 | 20 | 32 |
| | 2 | 22 | 30 |
| | 3 | 28 | 24 |
| May 13 | 4 | 31 | 64 |
| | 5 | 43 | 66 |
| | 6 | 57 | 44 |
| July 14 | 7 | 19 | 25 |
| | 8 | 55 | 129 |
| | 9 | 63 | 82 |
| Aug. 12 | 10 | 10 | 55 |
| | 11 | 17 | 80 |

[a] Each experiment involved a pair of sections, one cut from a portion of a branch showing bark lesions of psorosis, the other from an unaffected portion of the same branch or set of branches. Experiment conducted as shown in figure 5.

Numerous experiments on the passage of water through psorosis-affected and nonaffected wood were then carried out. In one series of experiments, sections about 5 cm. long were cut from Valencia-orange branches 2 to 3 cm. in diameter. Prepared sections were paired, one section of each pair being from a portion of the branch showing a bark lesion of psorosis A, and the other section from a portion having no bark lesions. A glass tube 2.2 cm. in diameter was sealed to one end of each section with Duco cement, and the other end was placed in water. The same suction was applied simultaneously to the glass tubes by means of a U tube, as shown in figure 5. The time required for water to pass through the sections and to rise to a 10 cc. mark

was then recorded. The time is shown for 11 pairs in table 3. In some of the experiments where suction was not applied simultaneously, the affected and nonaffected sections of the pairs were tested under approximately the same conditions of pressure, the test of one section quickly following that of the other.

In these tests, with two exceptions, water passed through those sections having bark and primary wood lesions but no indication of secondary lesions,

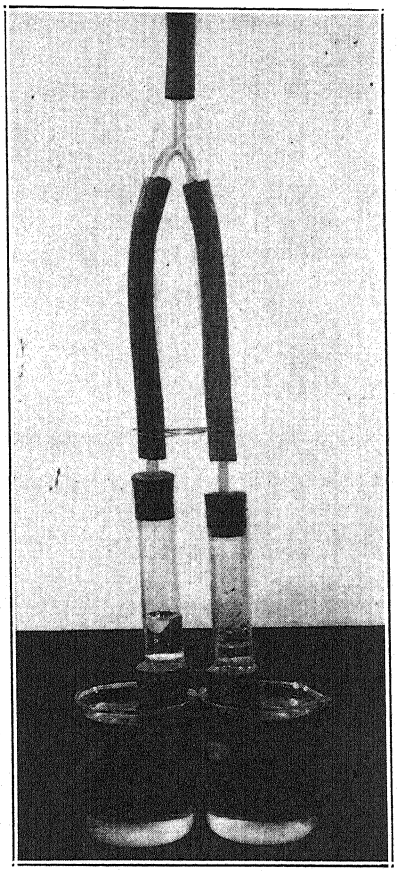


FIG. 5. Apparatus for drawing water through sections of healthy (left) and diseased (right) branches, in pairs, under the same suction. Note difference in water level under same suction.

somewhat more slowly than through corresponding sections cut from the same branch or set of branches, but without bark lesions. Although this indicates a tendency for water to be retarded in the wood vessels adjacent to or under the bark lesions, the retardation of water, obviously due to the primary lesion alone, is insufficient in most cases to cause apparent injury to foliage and fruit. This agrees with most field observations, that no marked deterioration of the tree occurs, even with bark lesions present, so

long as no interior discoloration of wood, characteristic of secondary lesions, is present. The slight or partial retardation in water passage shown in table 3 probably results from the initial gumlike deposits between the outer layers of wood and from some gum plugs in wood vessels in the primary lesions. Sufficient normal or unaffected wood is laid down to allow enough water to pass to meet the requirements of the tree.

## Water Conduction in Wood Having Both Primary and Secondary Lesions

The following experiments with wood having secondary lesions of starch-free, gum-plugged wood indicate the main reason for rapid deterioration of the tree.

*Experiment 1.* Experimental material was taken from a psorosis-infected Valencia orange tree. Sections 5 cm. in length were cut from branches 2 to 3 cm. in diameter, showing severe interior discoloration (secondary lesions) in the wood in certain portions. Suction was applied, as in previous experiments, to each of 3 sections of a branch cut beyond the discolored wood and showing no secondary lesion. The time required for 1 cc. of water to pass through these blocks was 17, 22, and 28 seconds, respectively.

With 3 other sections, each having a secondary lesion showing advanced wood discoloration with a margin consisting of a narrow band of clear wood extending to the bark, it required 5 minutes for 0.2, 0.2, and 0.4 cc. of water, respectively, to pass through. At the same rate, it would have taken 25, 25, and 12½ minutes, respectively, for 1 cc. of water to pass.

In another comparison from the same tree, a block with no secondary lesions showed passage of 1 cc. of water in 41 seconds, but a block with partly discolored wood of a secondary lesion required about 4 minutes for the passage of 1 cc. of water. In these comparisons, the presence of secondary lesions in the wood retarded water passage sixfold to eighty-eight fold.

*Experiment 2.* Similar tests were conducted on wood from a Valencia tree affected with psorosis B (6). Under a suction of 60 cm. of mercury, in two separate tests, 1¼ and 1¾ minutes were required for 10 cc. of water to pass a distance of 11.5 cm. through branches 2.5 cm. in diameter, showing bark lesions and primary lesions of the wood. In a comparative test with a branch of the same dimensions with wood showing also the discolored secondary lesions, 26 minutes were required for passage of 4 cc. of water, a rate approximately 45 times slower than that obtained with the wood not showing secondary lesions.

This comparison of time of water passage between sections having secondary lesions of discolored wood and sections having only bark lesions and primary lesions, without discoloration in the wood, appears to explain why branches having secondary lesions suffer deterioration and final decline, especially in hot, dry weather. Such branches are unable to get water fast enough in times of stress to supply the foliage and fruits. Many leaves,

therefore, drop and some twigs die back until the smaller supply of water available can take care of the remainder.

*Experiment 3.* Cylindrical blocks of wood, 5 cm. high and 7.5 cm. in diameter, were prepared from healthy navel-orange trunks and from trunks having interior wood discoloration. Glass tubes about 1 cm. in diameter were sealed to the upper, cut surface of each block with Duco cement and plasticine. The portion of the block between the bases of the tubes was covered with Duco cement. These blocks were then tightly placed in the end of a glass cylinder, 7.5 cm. inside diameter, with an outlet at the top fastened to the suction hose.

With a cylinder of wood from a healthy tree, showing starch over the entire cross section, the time required for 1 cc. of water to pass, in 3 regions in the section near the bark, was 26, 42, and 53 seconds, respectively. With a cylinder of wood from a diseased tree, showing starch in corresponding regions near the bark but having a secondary wood lesion some distance inward, the time required was 30, 60, and 90 seconds, respectively. In regions in the diseased wood of the latter specimen, where no starch reaction was shown by the I–KI test, no water reached the upper surface in 15 minutes.

This experiment indicates that with portions of wood from a diseased tree, even though the wood may appear normal and show the starch reaction, the time required for water passage is somewhat longer than with similar portions of wood from a healthy tree. It also shows that in the discolored wood of secondary lesions of psorosis, water passage is entirely blocked.

*Experiment 4.* In this experiment, glass tubes about 1 cm. in diameter were sealed with Duco cement in 0.5-cm. holes bored into sections of trunk and limbs showing the discoloration of secondary lesions. Each specimen was cut so that there would be 5 cm. of wood for the water to pass through. The time required for 1 cc. of water to pass through 5-cm. lengths of wood, at different locations in three different specimens, is shown in figure 6.

The results show that, as in previous experiments, only in the wood where the I–KI reaction indicated the presence of relatively large amounts of starch did water pass through readily. In areas of discolored wood or in areas not reacting to I–KI, no water passed when suction was applied for 10 to 20 minutes. At the edge of the discolored area in specimen C, at *f* (Fig. 6), where the I–KI test showed the presence of starch in about one third of the area covered by the tube, only about 0.1 cc. passed in 5 minutes, but no water passed through the discolored areas at *g* and *h*. In specimen B (Fig. 6), which was cut beyond the discolored portion of the limb, 1 cc. of water passed a distance of 5 cm. in the starch-bearing region (*d*) in $1\frac{1}{2}$ minutes, but no water got through the starch-free wood (*e*) even after long periods of time. In specimen A (Fig. 6), from a branch of a healthy tree, with the original growth center much to one side, the rate of water passage was many times faster (1 cc. in 35 seconds), at *a*, on the side where the wood had grown more rapidly, than at *c*, on the other, slower-growing side (rate,

1 cc. in 17 minutes). Near the center, at *b*, the rate was 1 cc. in 24 minutes and 1 second.

*Experiment 5.* A cross section, 11 by 13 cm., of a 5-cm. block of wood cut from a trunk of a diseased tree is shown in figure 7. Water passed readily through the 5 cm. of wood at *a* and *b*, lying outside the margin of the psorosis-discolored area (1 cc. in 60 and 45 seconds, respectively). On the other hand, water either failed to pass, or else moved very slowly, through the wood closely adjacent to or within the secondary lesion of discolored wood. In one region (Fig. 7, *c*) half within and half outside the discolored

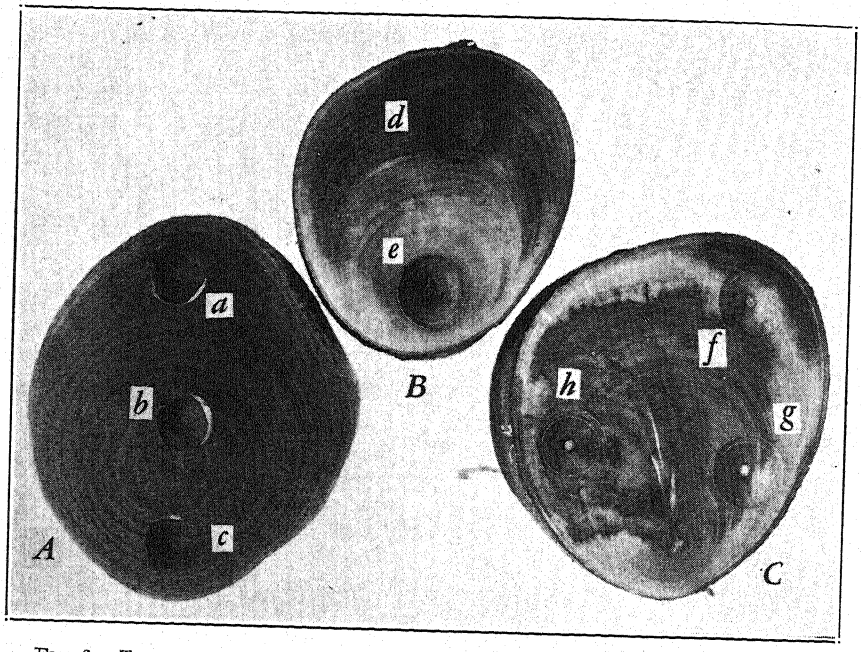



FIG. 6. Transverse sections of 5-cm. blocks of wood from Valencia-orange branches, on which graduated tubes had been attached for water suction. The regions showing the heavier black coloring are those containing starch, as indicated by the I–KI test. The central, irregularly colored region of section C is wood discoloration caused by psorosis A. A. Block from a healthy branch. The presence of starch is shown over the entire section, and water passed in all three regions, as follows: *a*, 35 seconds; *b*, 24 minutes, 10 seconds; *c*, 17 minutes. B. Block from a diseased branch, cut above but near discolored wood of secondary lesion. Starch was present only in the outer layers of wood on one side of the branch, where 1 cc. of water passed (*d*) in 1 minute, 30 seconds. No water could be drawn through the starch-free area (*e*). C. Block from the same branch as B, but containing psorosis-discolored wood. Wood containing starch was confined to a very small portion (upper right). It required 50 minutes for 1 cc. of water to pass through this region at *f*. No water passed through the other two regions (*g* and *h*) of discolored wood where no starch was indicated. All × ¾.

wood, it took 24 minutes for 1 cc. of water to pass. The regions *d*, *e*, *f*, and *g* (Fig. 7), occupied by the psorosis wood lesions, allowed no water to pass in 24 minutes or more, under the same suction.

The results in experiments 4 and 5 (Figs. 6 and 7) show, further, that the passage of water appears to be completely prevented in the region of the

discolored secondary lesions. They also show, as have some of the previous experiments, that outside of the discolored region, absence of starch appears to be coincident with the regions where passage of water is partially or totally blocked. This blocking of the supply of water would, in part at

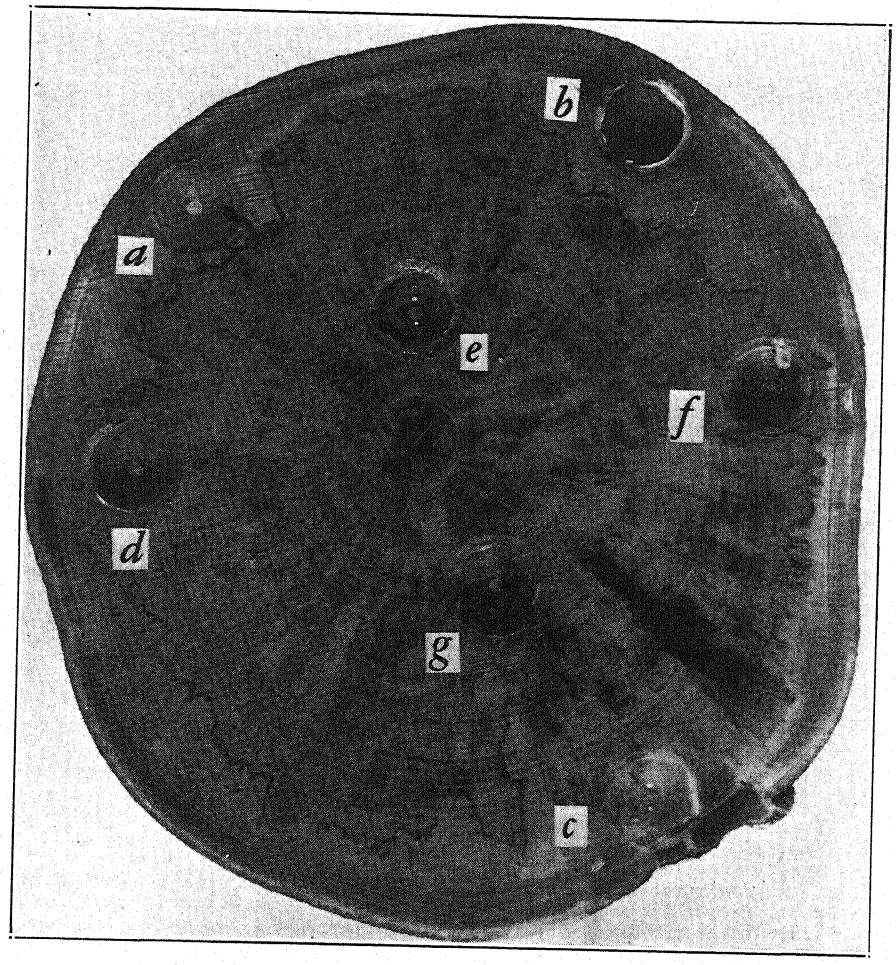



FIG. 7. Cross section of a block of wood from a diseased Valencia-orange trunk cut beyond the bark lesion but showing severe secondary lesions of the wood. The time required for 1 cc. of water to pass, under a pressure of four-fifths of an atmosphere, through this 5-cm. block, in which the I–KI test showed the presence of some starch, was as follows: *a*, 1 minute; *b*, 45 seconds; *c*, 24 minutes. These three regions were partially or wholly outside the psorosis discoloration. In four other regions (*d*, *e*, *f*, *g*), all within psorosis-discolored wood, no water could be drawn. The top of the tree from which this block was cut had only partially deteriorated.

least, account for the gradual deterioration and decline of trees having well-developed secondary lesions in the wood, and would account for the rapid deterioration where the secondary symptoms involve more nearly the entire wood in some part of a trunk or limb.

AIR PASSAGE THROUGH BARK

The question also arose as to the possible effect of psorosis lesions on the amount of air that can pass through the bark. A simple experiment showed that more air passed from the wood through the area occupied by the bark lesion, especially at the margin, than through the uninvolved bark just beyond.

Several branches, about 2.5 cm. in diameter and 15 cm. in length, were used in these experiments. Each branch showed a bark lesion of psorosis A completely girdling one of its extremities. The affected end was carefully sealed with an air-tight rubber cap. The other end was sealed into a glass

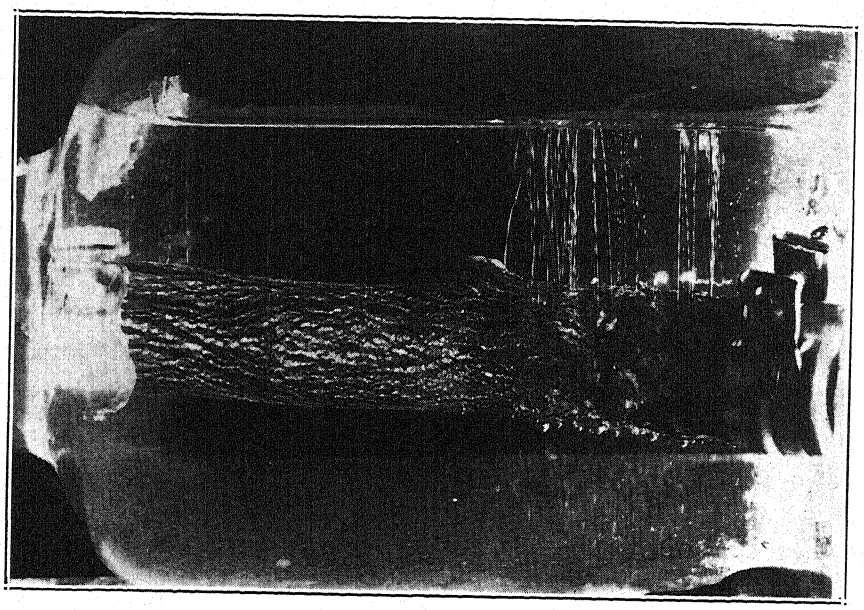


FIG. 8. Section of a citrus branch, with a psorosis bark lesion completely girdling one of its extremities. The affected end of the branch (right) is sealed with an airtight rubber cap; the unaffected end (left) is sealed into one end of a glass tube, which extends through the rubber stopper of the glass jar. The branch is immersed in 95 per cent ethyl alcohol. Suction in the air space above the alcohol was maintained equivalent to 60 cm. of mercury (about four-fifths of an atmosphere). Streams of air bubbles are seen arising chiefly from the psorosis-affected bark and escaping at the surface of the alcohol. × §.

tube, which was passed through the rubber stopper of the glass jar. One cut end was thus exposed to the outside air. The branch was placed in the jar, and the stopper was adjusted. The jar had been partly filled with 95 per cent ethyl alcohol and was maintained in a horizontal position during the experiment (Fig. 8). Through a bent glass tube also passing through the rubber stopper, the air was pumped out of the space in the jar above the alcohol so as to maintain a difference in pressure equivalent to 60 cm. of mercury (about four-fifths of an atmosphere).

At first, a great many minute air bubbles moved up from the whole surface of the branch tested, especially if it had partially dried before the

beginning of the experiment. After 5 to 10 minutes, the streams of bubbles could still be seen to proceed from the diseased bark, mainly from the margin of the lesion, while only a few, or none, escaped from the unaffected bark.

In one experiment, for example, only 6 small streams of bubbles were observed to proceed from the unaltered bark, which occupied a length of 6 cm. Because of their great number, it was difficult to count the streams of bubbles arising from the psorosis bark lesion, which occupied a length of about 3 cm., but more than 30 were observed. The bubbles from the diseased portion of the branch were larger than those from the unaffected portion. In the older part of the lesion, occupying 0.5 cm., only 3 or 4 streams of bubbles were apparent. This was probably due to the formation, in this area, of new bark whose permeability was similar to that of unaltered bark.

In figure 8 the streams of bubbles are clearly shown against a black background. Here most of the streams of bubbles may be observed to come from the margin of the lesion between regions of severe scaling and unaffected bark. This is to be expected, since this is the most recently disturbed region of the bark. The experiments were run for several hours without appreciable change in the number or size of the bubbles.

When a young, healthy branch was used in an experiment similar to the one shown in figure 8, using water instead of ethyl alcohol, numerous bubbles were seen to form on the surface of the bark. These bubbles were arranged longitudinally along the lenticels, and apparently no bubbles formed on the portions away from the lenticels. The bubbles remained attached to the point of formation for a considerable period of time and were observed to become detached when they approached a size of about 0.25 mm. in diameter. The time necessary for a bubble to attain this diameter varied considerably. Those forming most rapidly remained attached about 15 minutes. The majority, however, remained attached several hours.

In order to determine which tissues retained most of the air, an experiment similar to the one shown in figure 8 was performed with a healthy branch immersed in water. A circular band, 1 cm. wide, was carefully so scraped around the branch as to remove only the epidermis and the lenticels and leave as much of the cortical parenchyma as possible. On another portion of the branch, the whole bark was removed around the branch and down to the wood for a space approximately 1.5 cm. in width. The branch was then placed in water in a jar, as in the preceding experiment, and suction was applied.

As shown by the air bubbles, considerable amounts of air passed through the bark which had been scraped lightly, while very few air bubbles arose from the band of exposed wood (Fig. 9, A). The bubbles from the scraped bark of the healthy branch were much smaller than those from the unscraped, psorosis-affected bark in the earlier experiment, but the number of streams was greater. From observations of bubbles shown in figure 9, A, it was apparent that most of the air passed from the exposed, cut end of the branch had come in through the wood vessels and then by means of the

medullary rays had passed to its points of escape from the bark. It was, therefore, suspected that the reason why no air passed from the surface of the exposed wood was that during the girdling operation the cambium had been exposed to air and had thus been rendered impervious to air, possibly through clogging of the medullary rays as a result of coagulation of the cytoplasm. The branch, therefore, was taken out of the glass jar, and a new band of bark was removed beyond the already peeled portion, the operation this time being performed under water. When suction was again applied above the surface of the water in which the branch was immersed, a comparatively large amount of air escaped at the point where the wood had

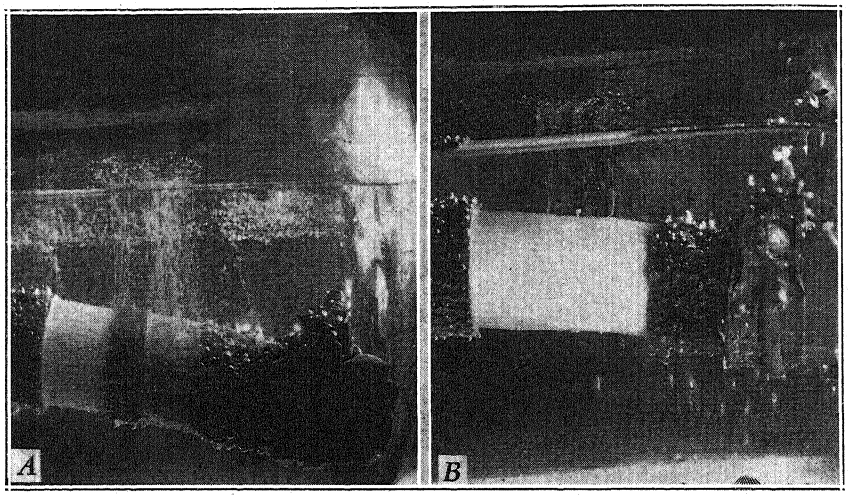



FIG. 9. Air-suction experiment on a section of a healthy citrus branch immersed in water in a glass jar similar to that shown in figure 8. A. Branch girdled by a superficial scraping of the bark (right) and by a peeling away of the bark in the dry air of the laboratory (left). Streams of air bubbles are seen to proceed almost entirely from the scraped area, practically no air being drawn from the exposed wood except at the edges where the vessels were cut. B. The same branch with peeled girdle enlarged by further peeling, under water. Numerous streams of bubbles are now seen to proceed from the newly exposed wood. × about 5/9.

been exposed by removal of the bark under water (Fig. 9, B); thus the hypothesis was confirmed.

As may be seen on the surface of citrus branches, the lenticels form long longitudinal strands of grayish tissue anastomosed laterally and separated by elongated dull-green areas where the epidermis is continuous. The dull-green color is due to the chloroplasts in the underlying parenchyma. In anatomical sections it may be seen that the lenticels correspond exactly to the larger medullary rays, whereas the dull-green areas, still covered with the epidermis, are immediately above the bundles of pericyclic fibers. The lenticels are, therefore, separated from the wood only by the living cells of the bark parenchyma, the medullary rays in the phloem, the cambium, and the medullary rays in the wood. It is obvious that the air passage from the

lenticels to the wood vessels takes place entirely along those tissues of living cells.

By scraping the surface of the bark of a psorosis lesion, the characteristic brown, irregular areas of gummy bark are exposed. If suction is applied to the surface of the water in which a branch thus treated has been immersed, streams of bubbles proceed mostly from the margin of the brown areas. Streams of bubbles of smaller diameter are also seen to proceed from the portions of the lesion that are still green, while none, or very few, come from the brown regions themselves.

In another, similar experiment with a branch 2.5 cm. in diameter and 11.5 cm. long, showing psorosis-B lesions and afterwards found to have a secondary lesion, small streams of air bubbles came from the entire surface of each bark lesion—possibly about the same total amount of air as from equal-sized lesions of psorosis A. A corresponding healthy branch showed less air emerging under the same pressure.

In another series of experiments with wood from a tree infected with psorosis A, disks of bark, 3 cm. in diameter, were sealed to one end of glass tubes and immersed in alcohol over which a suction equivalent to four-fifths of an atmosphere was maintained, as in the previous experiments. No air bubbles were observed in short-time tests (about 5 minutes) with 3 specimens of unaffected bark taken not far from lesions of psorosis. In a similar test with 3 specimens of affected bark from lesions, 0, 2, and 3 streams of bubbles were counted, respectively. The bubbles were approximately 0.2 cm. in diameter. In these tests, in which the bark was without attached wood, the air passed directly from the atmosphere through the inner bark and on to the outer bark and to the alcohol in which the specimens were immersed. Comparable results were obtained also in tests with both healthy and diseased bark, using disks 1 cm. (instead of 3 cm.) in diameter.

From the foregoing experiments, it is concluded that (a) bark with psorosis lesions will allow the passage of considerably larger amounts of air than normal bark; that (b) in normal bark the air passes through the lenticels only; that (c) the epidermis between the lenticels constitutes the principal barrier preventing air from passing freely through the bark; and that (d) most of the air passing through the psorosis lesions comes through minute openings largely located on the margin of the lesions, and more especially on the margin of the brown, gummy areas of the lesions.

## DISCUSSION AND CONCLUSIONS

It is known that bark scaling and primary-wood lesions, with certain minor alterations of wood, may be present for a long time in psorosis-affected trees without causing a great amount of tree deterioration. Results of the present study indicate that at these earlier stages of the disease, the passage of water through the wood vessels is only partly impeded; and the amount of starch present in the wood cells is about the same as that in the wood cells of healthy trees. This appears to explain the fact that trees may toler-

ate bark and primary wood lesions for some time with slight or imperceptible decline.

In later stages of the disease, however, when the secondary symptoms appear, that is, when discoloration of the interior wood occurs, water cannot pass through this discolored region or through contiguous regions, and can pass only with great difficulty through wood some distance away from the discolored region. The I–KI starch test, as well as microscopic sections, have shown that all the parts impermeable to the passage of water are devoid of starch, and that the wood vessels in these parts are clogged with gum or a gumlike material.[6] Even in such cases, however, limited regions of wood at various places near the outside of the tree, next to the bark, will be found to conduct water fairly readily. Such areas correspond to regions showing starch reactions with I–KI. It appears that the gradual extension of the regions in the wood in which water passage is blocked may account for the gradual deterioration of the trees.

It was first thought that perhaps the initiation of the discoloration of the wood accompanying the old bark lesions might be related to the passage of air and other gases in and out of the wood by way of the bark. Experiments showed that, under pressure, air passed more easily through the bark of the regions occupied by bark lesions, especially at the margins, than through normal bark. It is therefore concluded that lack of air is probably not responsible for initiation of the secondary lesion. Whether or not increased air has any relation to wood discoloration needs further investigation.

Although there appears to be a close relationship between the extent of the discolored wood and the decrease in water passage, it is possible that still other factors may play some part in the deterioration of the tree. Altered nutrition or toxic substances produced by the discolored-wood region may cause some injury to psorosis-affected citrus trees, but so far, no evidence has been obtained that such factors are important in this connection. That toxic substances are not an important factor is indicated by the observation that only the tree parts above and directly connected with the portion bearing the secondary lesion show deterioration. It would be expected that if toxic substances were present, they would be transported to other parts of the tree and cause injury.

## SUMMARY

Two kinds of wood lesions accompany and follow the bark lesions of the two varieties of psorosis virus, psorosis A and psorosis B, on citrus: (a) primary lesions, immediately under the bark lesions, in which gum is formed between outer layers of either normal or altered wood; and (b) secondary lesions, in which discoloration appears to begin in older wood farther inward, and in which starch disappears and the vessels become plugged with gum.

Primary lesions were found to retard water passage only partially.

[6] Tyloses were also looked for but were not noted. Water appears to pass, to some extent, through old, healthy citrus wood.

Their presence usually did not prevent almost normal development of foliage and fruit. In the discolored region of the secondary lesions, however, and in a starch-free region contiguous to the visibly discolored region, the passage of water was completely stopped. Both of these regions were found to be devoid of starch, as indicated by the I–KI test for starch.

The progressive degree of stoppage of water as the secondary lesions increase in extent and lessen the amount of normal water-conducting wood, is considered the main factor in the deterioration of trees affected with psorosis. Impaired nutrition or toxins may also play a small part.

In experiments with air, under a difference in pressure of approximately four-fifths of an atmosphere, it was found that air passed only slowly through normal bark, and only in the regions of the lenticels. Much more air passed at the bark lesions, especially near their margins, than at areas contiguous to these lesions. This indicated that the wood discoloration and the accompanying deterioration of the tree were not caused by lack of air passage at the bark lesions. Lack of water conduction, rather than lack of air passage, appears to be the principal factor in tree deterioration from psorosis.

UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA.

LITERATURE CITED

1. ANDERSON, B. E., R. A. GORTNER, and HENRY SMITH. Factors affecting the decreased rate of flow of liquids through wood. Minnesota Agr. Exp. Stat. Tech. Bull. 146: 1–70. 1941.
2. BARTHOLOMEW, E. T. Internal decline (endoxerosis) of lemons. V. Concerning the comparative rates of water conduction in twigs and fruits. Amer. Jour. Bot. 15: 497–508. 1928.
3. FAWCETT, H. S. Citrus diseases and their control. 2d ed. 656 p. McGraw-Hill Book Company, Inc. (New York). 1936.
4. ————. Suggestions on plant virus nomenclature as exemplified by names for citrus viruses. Science 92: 559–561. 1940.
5. ————. Citrus viruses. Phytopath. 31: 356–357. 1941.
6. ————, and A. A. BITANCOURT. Comparative symptomatology of psorosis varieties on citrus in California. Phytopath. 33: 837–864. 1943.
7. VILLIERS, ETHELWYN DE. The xylem anatomy, relative water conductivity and transport of dyes in citrus stems. So. Afr. Jour. Sci. 36: 291–313. 1939.
8. WEBBER, IRMA E., and H. S. FAWCETT. Comparative histology of healthy and psorosis-affected tissues of *Citrus sinensis*. Hilgardia 9: 71–109. 1935.

# POPULATION TRENDS OF PHYSIOLOGIC RACES OF PUCCINIA GRAMINIS TRITICI IN THE UNITED STATES FOR THE PERIOD 1930 TO 1941[1]

E. C. Stakman, W. Q. Loegering, R. C. Cassell, and Lee Hines[2]

(Accepted for publication March 8, 1943)

From studies of the annual prevalence and distribution of physiologic races of *Puccinia graminis tritici* in the United States for more than 20 years, it is possible to draw certain conclusions regarding population shifts. Preliminary results have been published (9, 10, 13, 14, 19, 20, 21, 22), and many general statements based on these results emphasize the fact that a variety of wheat may be resistant in one region and susceptible in another in the same year, or that a variety may behave differently in the same place in different years because of differences in the prevalance and distribution of physiologic races.

There have been such decided changes in prevalence of certain physiologic races in the United States since 1930 that it seems desirable to give the data for nine common races for 1930 to 1941, inclusive (Fig. 1). These data are presented to illustrate certain principles, as a summary of all available data would far transcend the limits of a short paper.

## SCOPE OF WORK AND METHODS USED

Attempt was made each year to obtain a random and adequate sample of rusted wheat from the entire United States, and some collections of rusted barley and wild grasses also were obtained. The number of collections for each year follows: 1930, 288; 1931, 361; 1932, 325; 1933, 294; 1934, 478; 1935, 787; 1936, 619; 1937, 1,025; 1938, 1,030; 1939, 735; 1940, 902; 1941, 804.

In general, collections were made from a random sample of fields, regard-

[1] Cooperative investigations between the United States Department of Agriculture and the Minnesota Agricultural Experiment Station. Paper No. 2066, Scientific Journal Series, Minnesota Agricultural Experiment Station.

Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, Official Project 165–1–71–124, Subproject No. 491. Sponsored by the University of Minnesota, 1941.

[2] E. C. Stakman, Chief, Division of Plant Pathology and Botany, University of Minnesota, and Agent, Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture; W. Q. Loegering, Agent, and R. C. Cassell, formerly Agent, Bureau of Entomology and Plant Quarantine; and Lee Hines, formerly Agent, Division of Barberry Eradication, Bureau of Plant Industry.

The writers are indebted to M. N. Levine and Ralph U. Cotter for assistance in determination of physiologic races at various times, and to Donald G. Fletcher for furnishing a large number of collections. Thanks are also due many other individuals for sending collections from time to time; unfortunately the list would be too long if all names were enumerated. Special thanks, however, are due various members of the Bureau of Plant Industry, U. S. Department of Agriculture, and to the personnel of the barberry eradication project of the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine. The encouragement and advice of Dr. H. B. Humphrey during the early years of the investigation are gratefully acknowledged.

less of the variety of wheat. For example, when rust was generally prevalent, a collection was made about every 20 miles along the roads being traveled. In some cases, however, special attempt was made to determine

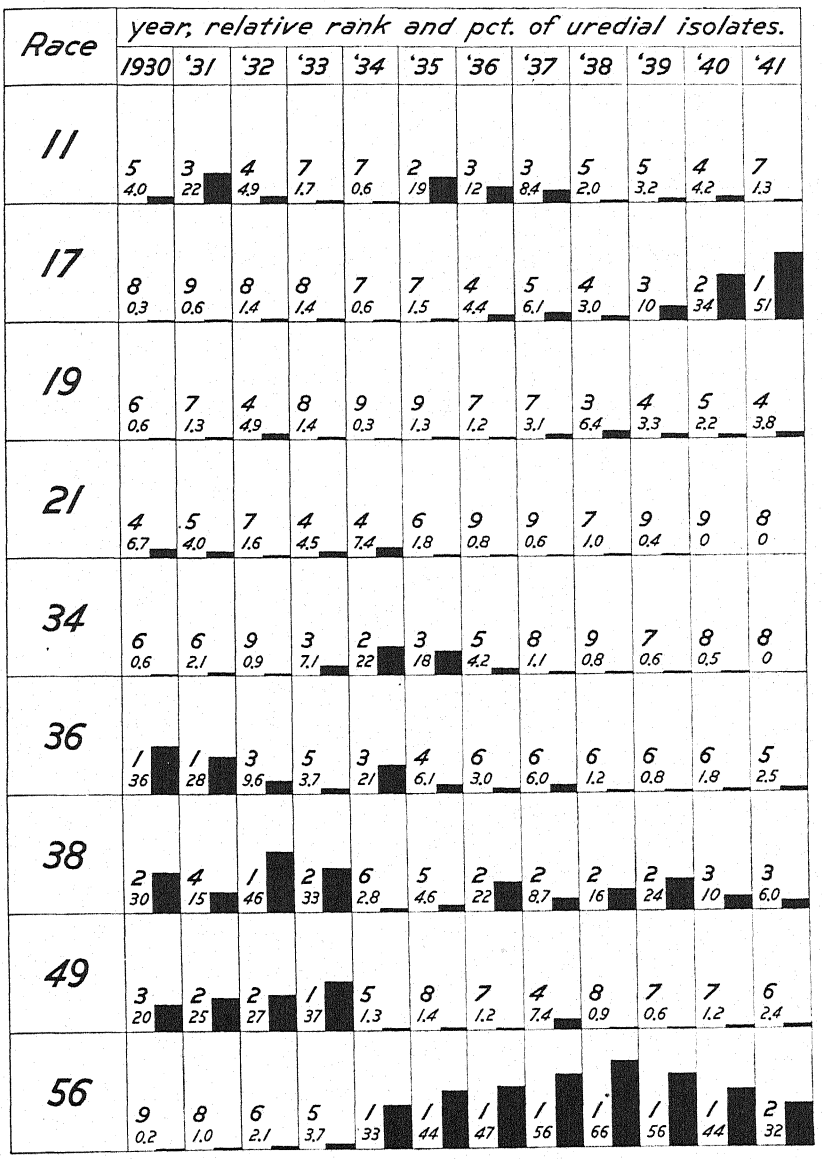



FIG. 1. Population trends of 9 physiologic races of *Puccinia graminis tritici* for the period 1930–1941, inclusive. Lower figure in each block indicates percentage of uredial isolates; upper figure, the relative rank in prevalence.

which races occurred on certain varieties, and it was necessary to depend upon volunteer cooperators for the rust collections in some areas; consequently there was some deviation from random sampling. Moreover, the

largest number of collections always was made in the wheat-growing area extending from Texas northward into the hard red spring wheat region and eastward into the soft red winter wheat area. Relatively few collections were obtained from the Pacific Coast and from the intermountain wheat-growing area of Washington and Oregon. The results, therefore, indicate the general prevalence of races for the country other than the wheat-growing regions west of the Rocky Mountains.

The percentage prevalance figures are an indication of the relative importance of races each year, although the distribution of races was not always completely uniform. Race 38, for example, was more prevalent in Indiana and eastward in some years than elsewhere; and races 56 and 17 were relatively scarce in those areas or localities where varieties resistant to them predominated. With exceptions such as these, there is abundant evidence that the percentage figures given in figure 1 indicate the relative importance of races by years for the country as a whole.

The collections were sent to the federal rust laboratory at University Farm, St. Paul, Minnesota, where races were identified by the methods described by Stakman and Levine (16).

In figure 1 are given the percentages of uredial isolates for each of 9 races for each of 12 successive years. The word "isolate" designates a race identified from a given collection, and its use is necessitated because more than one race often is isolated from a single collection. For example, races 56 and 11 commonly occurred in the same collections in 1935 (Fig. 2). Race 56 produced minute uredia and race 11 large ones on certain durum varieties; therefore, it was possible to identify the races definitely by making appropriate inoculations from the two kinds of uredia. The race obtained from each kind of uredia is an isolate, and in this case two isolates were obtained from one collection. Similarly, there often are two infection types, 2 and 4, on Marquis wheat. By making transfers to certain differential wheat varieties from type-2 uredia and from type-4 uredia, the races can be identified; and each is designated as an isolate. This is explained more fully and illustrated in a report for 1939 (19). Accordingly, the percentages given in figure 1 are based on the total number of times a given race was identified (isolated) in proportion to the total number of times all races were identified; they do not represent the percentage of collections from which each race was identified.

The prevalence of races expressed in terms of percentage of total isolates is, of course, only a relative indication of the situation. The percentage of collections from which each race was isolated may give a better idea of the geographic distribution. The following example is given by Stakman and Loegering (19) in a summary of the results of the survey in 1939:

> Consider race 56. . . . It was identified 590 times, and a total of 1,063 identifications were made of 14 races in 735 collections; that is, there were 1,063 isolates from 735 collections. Accordingly, race 56 constituted 55.5 per cent of all isolates. However, it should be remembered that the percentage figures . . . might be computed on the basis of number of field collections rather than number of isolates. For example, race 56 was isolated from 590 collections of stem rust out of a total of 735 collections, or from 80 per cent of the collections.

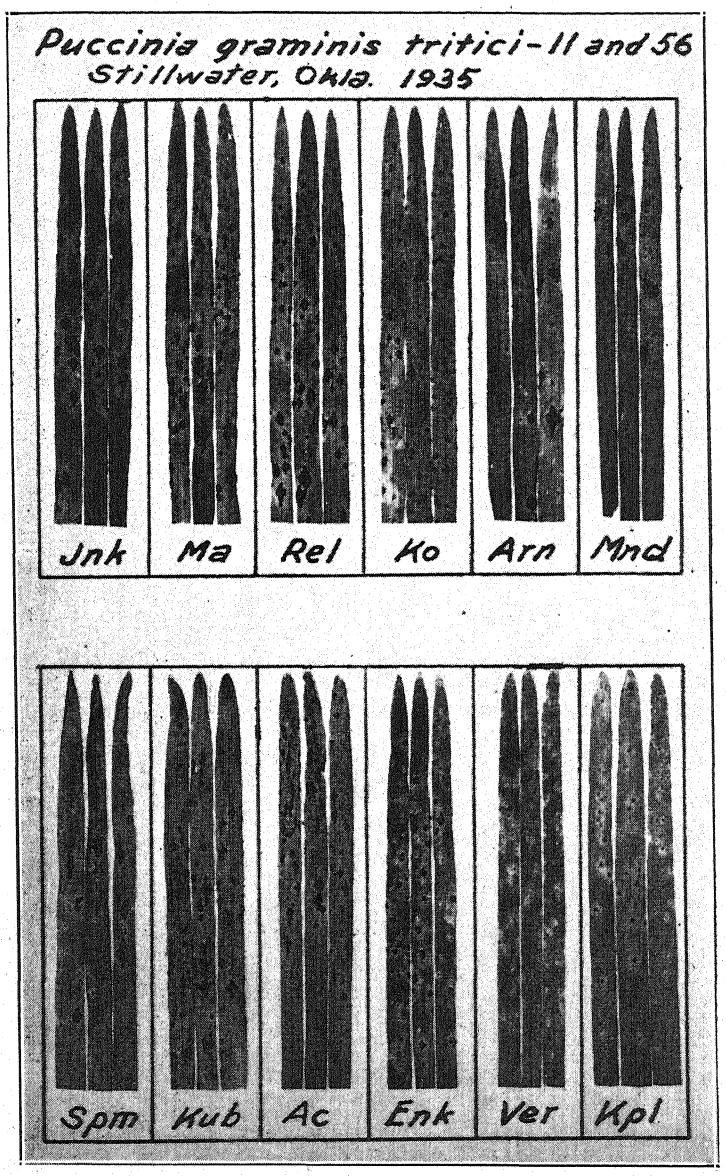



FIG. 2. Differential varieties of *Triticum* spp. inoculated with a collection of rust from Stillwater, Oklahoma, in 1935. This was typical of the infection types produced by many collections of rust in that year. The infection is caused by races 56 and 11. No distinction can be made between them on the first four varieties or on Kubanka, Acme, Vernal, and Khapli. On Arnautka, Mindum, Spelmar, and Einkorn, on the other hand, race 56 causes flecks or type–1 pustules, while race 11 produces type 3–4. There are about a dozen large uredia (caused by race 11) on Arnautka, Mindum, Spelmar, and Einkorn, but there are about 50 flecks or type–1 pustules (caused by race 56) on each of these varieties—all of which may not show in the reproduction. Obviously, therefore, in the original collection, inoculum of races 11 and 56 was present in an approximate ratio of 25: 100. Accordingly, each race comprises 50 per cent of the isolates, but race 56 is by far the more abundant.

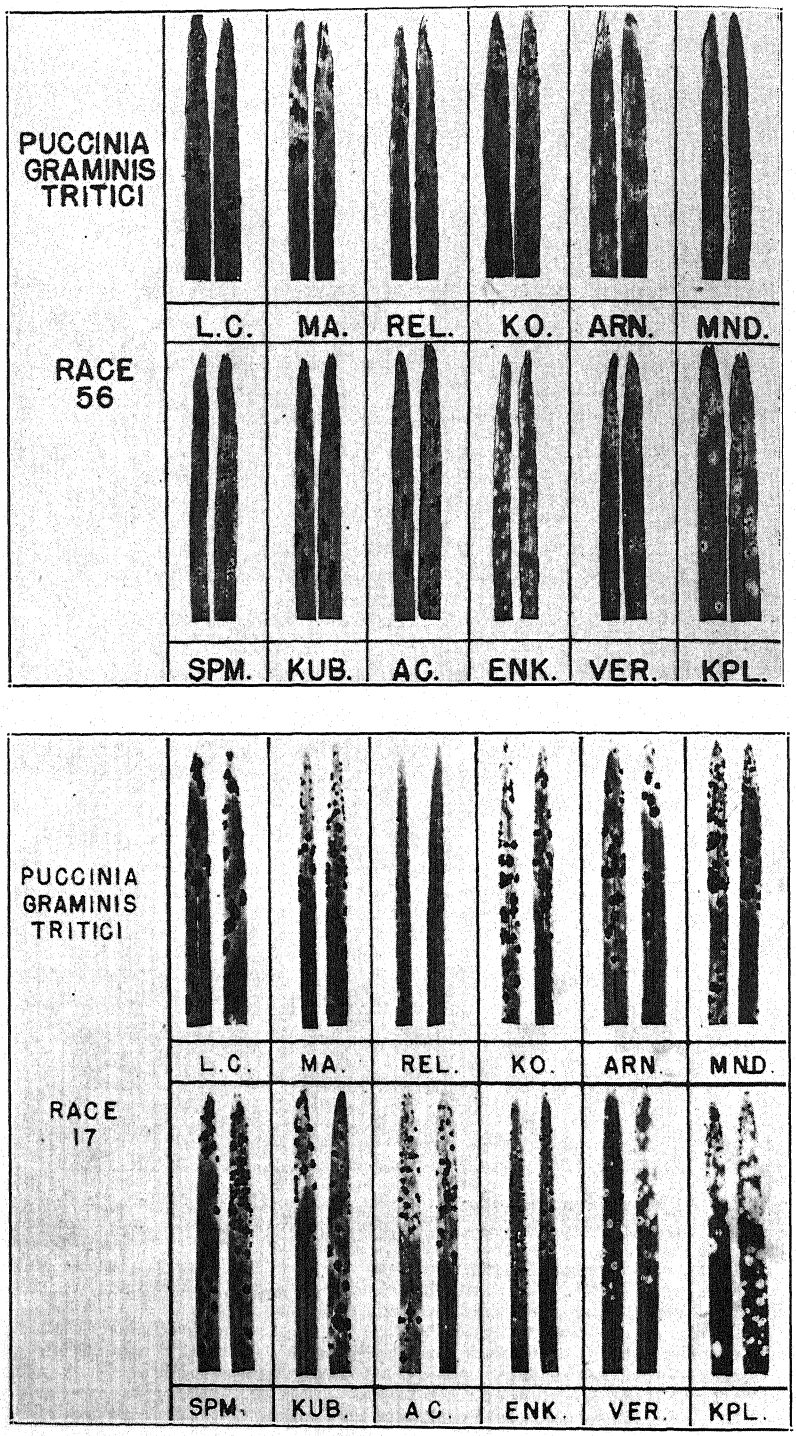



FIG. 3. Differential varieties of *Triticum* spp. inoculated with *Puccinia graminis tritici*, races 56 and 17. Note differences in infection types. Reliance (substitute for

But neither of these percentage figures is entirely adequate because the relative amounts of rust of different races in each collection also are necessary in estimating the importance of each race in producing infection in the field. Results obtained in 1941 furnish a good example (21). Races 17 and 56 were found together in many collections. The infection types produced by these races on the differential wheat varieties are illustrated in figure 3 and are given in table 1.

TABLE 1.—*Infection types produced by races 56 and 17 on differential varieties of Triticum spp.*

| Race | LC[a] | Ma | Krd | Ko | Arn* | Mnd* | Spm* | Kub* | Ac* | Enk | Ver | Kpl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 56 | 4 | 4 | 4 | 4 | 1 | 1 | 1 | 3+ | 3+ | 1 | 1 | 1 |
| 17 | 4 | 4 | 0 | 3+ | 4 | 4 | 4 | 4 | 4 | 3 | 1 | 1 |

* Durum wheats.

[a] The names of the varieties follow in the order given above: Little Club, Marquis, Kanred (Reliance often substituted), Kota, Arnautka, Mindum, Spelmar, Kubanka, Acme, Einkorn, Vernal, Khapli.

Type 0 indicates immunity; type 1, very resistant; type 3, moderately susceptible; type 4, very susceptible.

Kanred is susceptible to race 56 and immune from race 17, but Einkorn and three of the durums, Arnautka, Mindum, and Spelmar, are resistant to 56 and susceptible to 17. As attempt is made, except for special purposes, to obtain a random sample of rust in the fields from which collections were made and to inoculate the differential varieties with a random sample of the rust in the collection, the infection types characteristic of each race should appear on the differentials in about the same ratio as that between the numbers of spores of each race in the collection. In many collections made in 1941 the ratio of race 17 to race 56 was 85:15, 90:10, or even 95:5. For example, a 90:10 ratio is arrived at as follows:[3] Assume that there are 10 uredia on each leaf of those varieties susceptible to both races, but only one uredium on Kanred. On Einkorn and the 3 durums, Arnautka, Mindum, and Spelmar, on the other hand, there are 9 large uredia and a single minute uredium or fleck on each leaf. If transfers are made from the rust on Kanred or from the minute uredia on Einkorn or the 3 durums, race 56 will be obtained; and from the large uredia on Einkorn or on the 3 durums, race 17 will be obtained. If this is the average ratio for many collections, then each race occurs in 100 per cent of the collections and each comprises 50 per cent of the isolates. But the number of spores, potential inoculum in the field, for races 17 and 56 is in the ratio of 90:10. Clearly, then, race 17 would be the more important. In reality, however, there usually is fairly close agreement between percentage of collections, percentage of isolates, and actual amount of rust, especially for predominant races.

Kanred) is susceptible to race 56 and immune from race 17, while Arnautka, Mindum, Spelmar, and Einkorn are highly resistant (almost immune in the field) to race 56 and susceptible to race 17. (See table 1 for names of differentials.)

[3] A description and diagram of the method of estimating these ratios is given in the report of the race survey for 1939 (19).

For example, in certain years race 56 might almost have been the only race present for practical purposes, as it ranked first in prevalence by a wide margin, by both methods of computation, and constituted 85 to 90 per cent of the rust inoculum in many regions of the United States (12). Nevertheless, it would be desirable, if time and facilities permitted, to make computations designed to express the relative importance of races in terms of amount and distribution of inoculum.

These facts have an important bearing on the number of replications necessary to testing wheat varieties for rust resistance in the absence of artificially induced epidemics in which known races are used. Clearly, the amount of inoculum of the various races in given years and regions is a basic factor in determining whether the varieties have been adequately tested, assuming that weather conditions were favorable for rust development.

## RESULTS

Several facts become immediately apparent on inspecting figure 1. Some races were notably prevalent early in the 12-year period and then declined; others have increased from small beginnings; others have fluctuated considerably; and one, race 34, increased gradually in prevalence, and then decreased just about as gradually.

Race 56 has had the most spectacular career. A brief statement regarding this race was published in 1938 (9). It was first identified in the United States in 1928 and increased slowly in prevalence for 6 years, then suddenly assumed first rank in 1934 and continued to increase until it reached its peak in 1938. In 1939 it began to decline and continued to do so through 1940 and 1941; nevertheless, for 7 successive years, 1934 to 1940, inclusive, this race occupied first rank, usually by a wide margin. How long the decline, which began in 1939, will continue is a question, but the drop to 32 per cent in 3 years from the high of 66 per cent in 1938 certainly indicates a definite downward trend.

Races 36 and 49 are in direct contrast with race 56: they declined as race 56 increased. Race 36 was the most prevalent of all races in 1930 and 1931, declined in the next 2 years, then increased again in 1934, but since that time it has not been sufficiently prevalent to be really important. Race 49 increased for the first four years of the decade beginning with 1930 until it attained first rank in 1933; in 1934 it decreased sharply, and since that time it has been relatively rare except in 1937.

Races 11 and 38 have fluctuated considerably, without displaying definite trends.

Race 34 comprised 0.6 per cent of all isolates in 1930, increased until it attained second rank in 1934, with 22 per cent of all isolates; but since then it declined each year and was not isolated at all from uredial material in 1941.

The history of race 17 is significant. It was common in the first half of the decade 1920 to 1930, then decreased until it reached a low of 0.3 per

cent of all isolates in 1930 [unpublished data and Wallace (24)], after which it tended to increase slowly and somewhat irregularly. During the last 3 years it increased sharply and surpassed race 56 in 1941, attaining first rank, with 51 per cent of all isolates.

Races 17 and 56 together comprised most of the inoculum of stem rust in 1940 and 1941, making up 78 per cent and 83 per cent of all isolates in the two years, respectively. In both years race 38 occupied third rank in prevalence. In those years these 3 races, with 88 and 89 per cent, respectively, of all isolates, were the races to which wheats in general were exposed. No other races were sufficiently prevalent to be important practically, except in local areas. This illustrates the present tendency for a few races to predominate among the uredial isolates. This tendency is not nearly so pronounced, however, among aecial isolates, as shown by the data for 1940, which are given as an example.

A comparison of the data for uredial isolates and those for aecial isolates in 1940 is given in figures 4 and 5. Races 56, 17, and 38 occupied first, second, and third positions, respectively, among both groups of isolates. Race 36, however, was obtained much more frequently from aecial collections than from uredial collections; and this is true of race 34 also, which at one time had been very prevalent on wheat but which comprised only 0.5 per cent of all uredial isolates in 1940. It will be noticed that race 15, the most generally virulent race found in the United States, comprised 5 per cent of the aecial isolates in 1940, but was obtained in only 1 per cent of the uredial isolates.

The importance of barberries in producing and perpetuating races previously has been discussed by Stakman, Levine, Cotter, and Hines (17), and has been summarized by Stakman, Loegering, and Cotter, (22) in the report of the physiologic race survey for 1940 as follows:

> Races 9, 10, 14, 24, 40, 55, 69, 77, 79, 83, 117, 121, 126, 140, 146, and 147 of *Puccinia graminis tritici* were isolated only from barberries or from rusted wheat in the area where barberries become rusted. Barberries definitely were responsible for the occurrence of these races in 1940, as they were not found at all in Mexico, Texas, or Oklahoma, from which urediospores can be disseminated in the spring. Regardless of the relative amounts of rust resulting from wind-blown urediospores from the far south and from aeciospores produced on barberries, it is apparent that more kinds of rust races come from barberries. Race 56 was known in the barberry area for at least five years before it appeared in Texas and became established in northern Mexico. The predominance attained by this race caused farmers to discard Ceres wheat, and there is the possibility of a similar history in the future with respect to some of the rarer rust races and some of the newer varieties of wheat.

The practical importance of population shifts in physiologic races has been alluded to (10, 18, 19, 20, 21, 22). It is worth emphasizing, however, that Ceres wheat, grown commonly in the spring wheat region after its distribution in 1926, is so susceptible to race 56 that it was virtually ruined by rust in 1935, the first favorable rust year after this race had become abundant, and it was again severely injured in 1937. Stakman and Cassell (9), Johnson and Newton (7), and Stakman, Loegering, and Cotter (22) have summarized evidence regarding the role of race 56 in eliminating Ceres

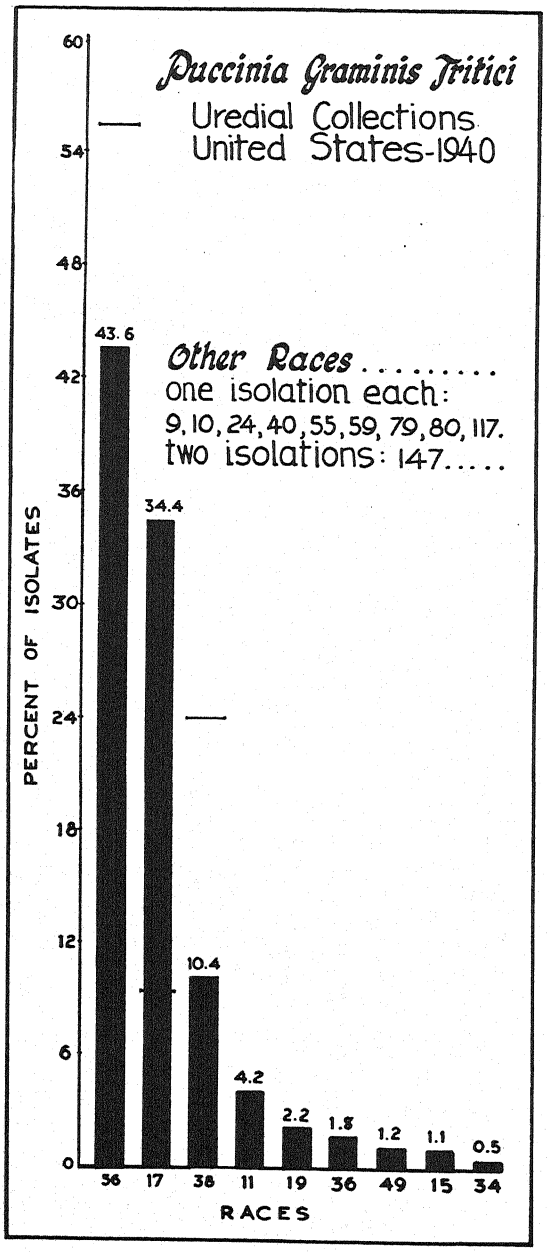



FIG. 4. Percentage of isolates of physiologic races of *Puccinia graminis tritici* obtained from uredial collections in 1940.

wheat. There is no question but that race 56 is more virulent than many other races on Ceres (Fig. 6). It often is possible to separate it from other races growing on seedling plants of Kota or Ceres in the greenhouse by simply making transfers from the largest pustules. So conspicuous is its

virulence on Ceres that an experienced observer can make a rather accurate guess as to its identity on differential hosts in the greenhouse merely by observing the size of the uredia on Ceres. Moreover, it seems particularly able to thrive on this variety at temperatures higher than those at which certain other races will thrive[4] (1). The tremendous increase in acreage of Ceres and a succession of unusually hot summers apparently favored the rapid increase of this race. Several other races might have eliminated Ceres had they become as prevalent as race 56; but the record shows race 56 responsible.

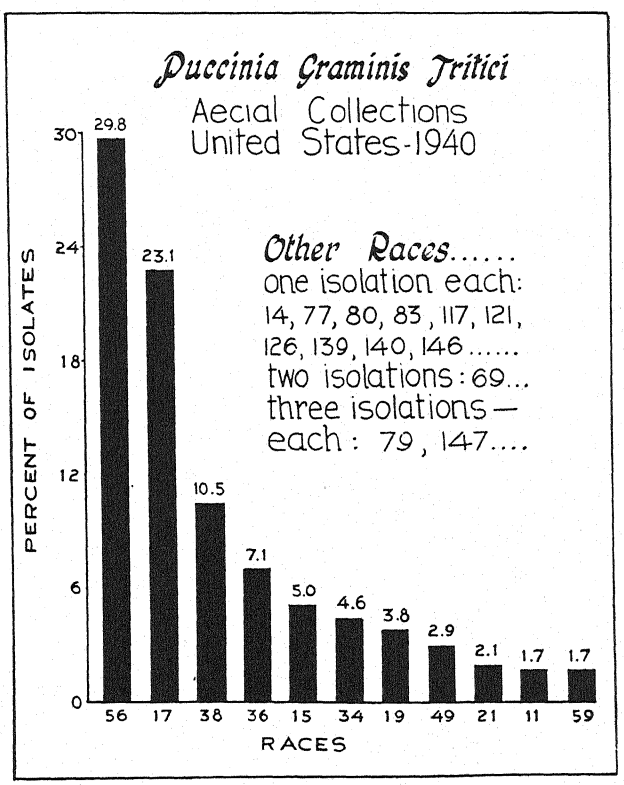



FIG. 5. Percentage of isolates of physiologic races of *Puccinia graminis tritici* obtained from aecial collections in 1940.

Thatcher wheat, first distributed in 1934, has very largely supplanted Ceres in the spring wheat area. It withstood the epidemic of 1935 so well that the acreage was increased greatly the next year. This variety is the result of a double cross (Marquis × Iumillo) × (Marquis × Kanred). It has the immunity of Kanred from a number of physiologic races and has considerable adult-plant resistance derived from Iumillo durum (3). Thatcher has not had a severe rust test under natural conditions, however, because it is highly resistant to or immune from those races that have been most

[4] Cassell, Robert C. Factors affecting the distribution of physiologic races of *Puccinia graminis tritici* Erikss. and Henn. University of Minnesota Ph.D. thesis. 1938.

prevalent since its introduction. Thatcher is moderately resistant in the seedling stage and highly resistant in the adult stage to race 56; and it is immune in all stages of development from races 17, 19, 21, and 49. Consequently, there has been a relatively small population of physiologic races to which it is susceptible. With the present predominance of races 17 and 56, Thatcher is still safe from serious damage. Nevertheless, it is susceptible to several races, including 36 and 15B, and has been heavily rusted in field plots.[5] If these races or still others to which Thatcher is susceptible become

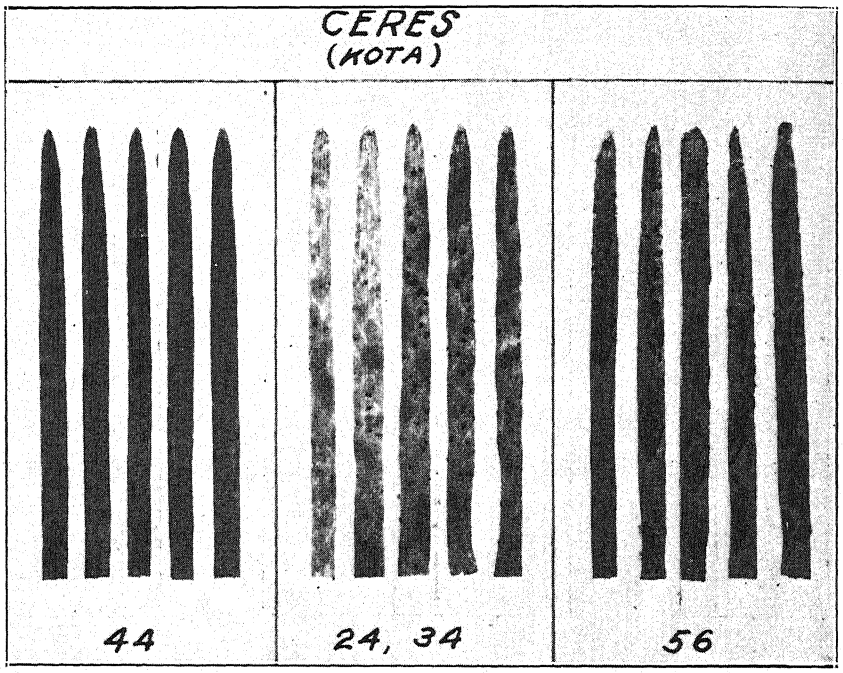



FIG. 6. Ceres wheat inoculated with 4 races of *Puccinia graminis tritici*, showing immunity from race 44, resistance to race 24 (two plants), moderate susceptibility to race 34, and extreme susceptibility to race 56.

prevalent and abundant, it is likely that it may be injured by rust when weather conditions favor rust development.

Race 17 was by far the most common race in much of the hard red winter wheat region in 1941. The behavior of wheat varieties was exactly what one would expect from the results of the physiologic-race survey. Tenmarq and other Kanred derivatives were relatively rust-free, whereas many of the soft wheats in neighboring fields were severely rusted. Similar observations were made in the spring wheat region. Thatcher was virtually rust-

[5] Thatcher was heavily rusted in replicated plots in the Cooperative Rust Nursery at St. Paul, Minn., in 1936. Race 36 was commonly isolated and seemed to be primarily responsible for the rust. Thatcher also has been heavily rusted in several recent years in field-plot experiments made by Helen Hart at St. Paul, the results of which have not yet been published.

free, whereas Marquis and Ceres, both susceptible to races 17 and 56, rusted rather heavily where conditions were favorable. Moreover, there was more rust on Mindum durum than on Thatcher.

The situation with respect to the durums, therefore, has fluctuated. Several years ago, when races 11, 21, 34, and 38 were prevalent, the most commonly grown durum varieties, especially Mindum, were severely rusted (15). During the past few years, however, when race 56 predominated, there was very little rust on the durums, except Kubanka, because most of those commonly grown are almost immune from this race. With the increase in race 17, however, they are again in jeopardy.

Although most varieties of wheat now grown may be injured more or less severely by certain races of rust, it must be emphasized that many of the varieties now available, especially in the hard red spring wheat group, are very much less liable to damage than the varieties that they replaced. Remarkable progress has been made in developing varieties with physiologic resistance to many races and with a certain degree of adult-plant resistance, due to morphologic and other characters, to most of the races. These varieties are, therefore, likely to remain resistant in more localities and in more seasons than those developed before there was sufficient recognition of the necessity for testing against many rust races under a wide range of environmental conditions in the breeding program.

Unfortunately, little is known definitely about the factors affecting changes in populations of physiologic races. It has been shown by several investigators[6] (1, 2, 4, 5, 6, 8) that environmental factors affect different races differently. Some of them develop best on certain varieties of grain at relatively high temperatures; others at low temperatures. The final amount of rust that develops on wheat depends on the physiologic race of rust, the variety of wheat, the effect of environment on each, and its effect on the interaction between the two. It seems likely, therefore, that seasonal temperatures may play an important part in determining prevalence of races. The prevalence of certain wheat varieties obviously has an effect also. Kanred, for example, which is immune from a number of races, would tend to decrease their prevalence over a considerable area. Likewise, if Mindum and similar durum varieties were grown exclusively in North Dakota, it would be quite impossible for race 56 to become prevalent there; and as long as Thatcher is grown in the spring wheat area there will be a tendency for it to reduce the prevalence of races 56 and 17 in that area and, consequently, the percentage prevalence in general.

Factors that affect the overwintering of various races in the uredial stage in northern Mexico and central Texas, the area in which uredial overwintering is most likely to occur, undoubtedly are important when considered in connection with the factors affecting the subsequent development of a large amount of inoculum in those areas and the widespread dissemination of spores northward. Fortuitous circumstances probably affect this

[6] See footnote 4.

situation somewhat, but there is evidence that certain races can overwinter more easily than others. Thus some may tend to be eliminated during the winter while others survive. Factors affecting the spread of the rust from these areas, therefore, would be important in determining the prevalence of the overwintered races in the country as a whole (23). Moreover, meteorological conditions affecting the dissemination of rust from areas where barberries become heavily rusted would also be important, as they appeared to be in 1942 (11). Although detailed results are not given for 1942, it is clear that a decided increase in prevalence of race 38 was due to the fact that it was produced commonly on barberries in the Virginias and Pennsylvania. Conditions were favorable for rust development in those areas, and for subsequent dissemination of inoculum westward into Ohio, Indiana, part of Illinois, and southern Michigan. While the prevalence of race 38 was rather high for the country as a whole, therefore, its prevalence was far higher in the States just mentioned than elsewhere.

## SUMMARY

Annual physiologic-race suveys of *Puccinia graminis tritici* have been made in the United States for more than 20 years. Results for 12 years are summarized in this paper, as they illustrate the decided shifts that may occur in the prevalence and importance of races.

During the 12-year period 1930–1941 some races have increased greatly, others have decreased, some have fluctuated irregularly, and one increased during the first half of the period and then decreased at about the same rate.

During the same period 5 races have ranked first in prevalence and amount in one or more years, namely, races 36, 38, 49, 56, and 17.

During the first half of the 1930–1939 decade races 36, 38, and 49 were the most important for practical purposes. During the last half of the decade race 56 was by far the most important, ranking first in prevalence for 7 successive years. Since 1939 races 56, 17, and 38 have been by far the most important and prevalent.

The meteoric rise of race 56, followed by a tendency to decline, and the decided increase of race 17 in 1940 and 1941 are especially significant. At present these two races appear to constitute at least 90 per cent of the inoculum in the Mississippi Basin.

Because of the spectacular rise in prevalence of race 56, beginning in 1934, Ceres wheat is no longer classed as resistant. With changes in the prevalence of races, it is possible, however, that Ceres might again attain to the resistant class.

During the past several years race 56 alone or in combination with race 17 has comprised between 80 and 90 per cent of the rust inoculum, at least in the spring wheat area. Thatcher, which has largely replaced Ceres, is resistant to race 56 and immune from race 17; therefore Thatcher has not yet been exposed to abundant infection, under favorable conditions, by a race or combination of races to which it is susceptible.

The population shifts of physiologic races show the practical need for extensive replication in time and space in testing varieties for stem rust resistance, unless the varieties are exposed to artificially induced epidemics created by inoculating with all races that occur or are likely to occur in the region for which the variety is intended.

Comparison of the number and kinds of races isolated from or near barberry bushes with those isolated away from barberries shows that the eradication of the alternate host as completely as possible is of paramount importance in reducing the number of races and preventing the production of new ones. Details for 1940, taken as a sample year, are given in the text.

The changes in relative proportions of individual races in the total population may be gradual or sudden; accordingly, the susceptibility and resistance of wheat varieties in the field tend to vary directly with these changes, although this tendency may be affected considerably by the additive effects of several races and by conditions that are favorable or unfavorable for rust development.

There is relatively little definite information regarding the causes for changes in prevalence of races, although seasonal temperatures, distribution of wheat varieties, conditions affecting the winter survival and subsequent development and dissemination of inoculum of different races from the far South, and the relative amounts of different races produced on barberries and disseminated from them all appear to be important.

UNIVERSITY FARM,
ST. PAUL, MINNESOTA

LITERATURE CITED

1. CASSELL, ROBERT C. Effect of temperature on infection and development of eight physiologic races of *Puccinia graminis tritici* on wheat seedlings. (Abstract) Phytopath. 29: 4. 1939.
2. ————. Effect of temperature on urediospore germination and germ tube development of five physiologic races of *Puccinia graminis tritici*. (Abstract) Phytopath. 29: 4. 1939.
3. HAYES, H. K., E. R. AUSEMUS, E. C. STAKMAN, C. H. BAILEY, H. K. WILSON, R. H. BAMBERG, M. C. MARKLEY, R. C. CRIM, M. N. LEVINE. Thatcher wheat. Minn. Agr. Exp. Stat. Bull. 325, 40 pp. 1936.
4. HWANG, LIANG. The effect of light and temperature on the viability of urediospores of certain cereal rusts. Phytopath. 32: 699–711. 1942.
5. JOHNSON, T. A study of the effect of environmental factors on the variability of physiologic forms of *Puccinia graminis tritici* Erikss. and Henn. Canad. Dept. Agr. Bull. (n.s.) 140, 76 pp. 1931.
6. ————, and MARGARET NEWTON. The effect of high temperature on uredial development in cereal rusts. Canad. Jour. Res., Sect. C, Bot. Sci. 15: 425–432. 1937.
7. ————, and ————. The predominance of race 56 in relation to the stem-rust resistance of Ceres wheat. Sci. Agr. 22: 152–156. 1941.
8. MELANDER, LEONARD W. Effect of temperature and light on the development of the uredial stage of *Puccinia graminis*. Jour. Agr. Res. [U.S.] 50: 861–880. 1935.
9. STAKMAN, E. C., and R. C. CASSELL. The increase and importance of race 56 of *Puccinia graminis tritici*. (Abstract) Phytopath. 28: 20. 1938.
10. ————, R. C. CASSELL, and W. Q. LOEGERING. Population trends of physiologic races of *Puccinia graminis tritici* in the United States from 1930 to 1939. (Abstract) Phytopath. 30: 22. 1940.

11. ————, R. U. COTTER, and W. Q. LOEGERING. Regional spread of wheat stem rust from barberry-infested areas of the Virginias in 1942. (Abstract) Phytopath. 33: 12. 1943.
12. ————, and L. M. HAMILTON. Stem rust in 1938. U. S. Dept. Agr., Pl. Dis. Reporter. Suppl. 117: 69–83. 1939.
13. ————, LEE HINES, ROBERT C. CASSELL, and M. N. LEVINE. Population trends of physiologic forms of *Puccinia graminis tritici*, 1930 to 1934. (Abstract) Phytopath. 25: 34. 1935.
14. ————, LEE HINES, HARRY G. UKKELBERG, and WALLACE BUTLER. Distribution of physiologic forms of *Puccinia graminis tritici* in the United States and Mexico in relation to rust epidemiology. (Abstract) Phytopath. 22: 25. 1932.
15. ————, ————, ————, and ————. Distribution of physiologic forms of *Puccinia graminis tritici* in relation to stem-rust epidemiology in 1932. (Abstract) Phytopath. 23: 34. 1933.
16. ————, and M. N. LEVINE. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Minn. Agr. Exp. Stat. Tech. Bull. 8. 10 pp., illus. 1922. (Mimeographed keys and tables for identifying races are available.)
17. ————, ————, R. U. COTTER, and LEE HINES. Relation of barberry to the origin and persistence of physiologic forms of *Puccinia graminis*. Jour. Agr. Res. [U.S.] 48: 953–969. 1934.
18. ————, ————, and J. M. WALLACE. The value of physiologic-form surveys in the study of the epidemiology of stem rust. Phytopath. 19: 951–959. 1929.
19. ————, and W. Q. LOEGERING. Physiologic races of *Puccinia graminis* in the United States in 1939. U. S. Dept. Agr., Bur. Ent. and Pl. Quar. E–522, 14 pp. (multigraphed). 1941.
20. ————, and ————. Recent changes in prevalence of physiologic races of *Puccinia graminis tritici* in the United States. (Abstract) Phytopath. 32: 17. 1942.
21. ————, and ————. Physiologic races of *Puccinia graminis* in the United States in 1941. U. S. Dept. Agr., Bur. Ent. and Pl. Quar. E–522-B, 11 pp. (multigraphed). 1942.
22. ————, ————, and RALPH U. COTTER. Physiologic races of *Puccinia graminis* in the United States in 1940. U. S. Dept. Agr., Bur. Ent. and Pl. Quar. E–522-A, 18 pp. (multigraphed). 1942.
23. ————, W. L. POPHAM, and ROBERT C. CASSELL. Observations on stem rust epidemiology in Mexico. Amer. Jour. Bot. 27: 90–99. 1940.
24. WALLACE, J. M. Physiologic specialization as a factor in the epiphytology of *Puccinia graminis tritici*. Phytopath. 22: 105–142. 1932.

# A COMPARISON OF LABORATORY AND FIELD RETENTION AND PROTECTIVE VALUE OF CERTAIN COPPER FUNGICIDES[1]

HAROLD J. MILLER[2]

(Accepted for publication February 4, 1943)

The need for reliable methods of testing fungicides is recognized by almost every worker in the field of plant pathology. The large amount of published data on this subject testifies to the demand for information on the fungicidal properties of materials before they are actually used in the field. The development of many new compounds, particularly organics and substitute copper materials to replace Bordeaux mixture, has stimulated the demand for methods that would more accurately evaluate the properties influencing the field performance of a fungicide.

Millardet and David (23), Girard (6), and Perraud (24) suggested very early that retention[3] might be an important factor influencing the protective value of copper fungicides. Kadow (14), Daines (2), Marsais and Segal (19), Hamilton (9), Martin (22) and Fajans (3) also have pointed out the importance of the retention factor in the performance of this type of fungicide. The early work of Guillon and Gouirand (8) on laboratory tests of retention also involved a correlation of this type of data with that obtained in the field. Quite comparable results were found on grape leaves and glass plates, where both surfaces were subjected to artificial rain and the amount of copper remaining was determined by chemical analysis. Kehlhofer (15) also reported some agreement between retention on glass plates and grape leaves as determined by chemical analysis. A good correlation between retention of certain copper fungicides, as determined by spore germination tests and by chemical analyses, was found by McCallan and Wilcoxon (16). Laboratory studies of retention of copper fungicides also have been reported by Heuberger (11, 12) and Young and Beckenbach (28), and field studies using chemical analysis by Magie and Horsfall (18) Hartmann (10) and Green and Goldsworthy (7).

The studies here reported involved the determination of the retention of certain copper spray materials by (a) direct chemical analysis before and after artificial rain on Pyralin plates and (b) chemical analysis of leaves before and after weathering in the field. The results of (a) and (b) were correlated with a third measure of retention (c), as determined by spore

[1] An abridgment of a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology in the Graduate School of The Pennsylvania State College, 1942. Authorized for publication on Oct. 7, 1942, as paper No. 1132 in the journal series of the Pennsylvania Agricultural Experiment Station, and as Contribution No. 138 from the Department of Botany.

[2] The author wishes to acknowledge his appreciation to Drs. D. E. H. Frear and H. W. Thurston, Jr., for their many helpful suggestions and criticisms during this study and to Dr. S. E. A. McCallan for determination of the tenacity indices and a reading of the manuscript.

[3] The term "retention" is used here as defined by the Committee on Standardization of Fungicidal Tests (1) in preference to "tenacity" or "adherence."

germination tests very kindly conducted by Doctor McCallan. Correlation of these measures of retentions with control data was determined for 1941. Toxicity determinations furnished by McCallan also were compared with this control data.

## METHODS

The method of laboratory spraying of cellulose nitrate (Pyralin) plates by atomizing on a rotating turntable has been described and illustrated by Worthley and Frear (27). For the retention studies an unwashed lot of 5 sprayed plates was designated as the original. Five were removed after being washed by atomizing with 0.25 l. of distilled water, 5 more after atomizing with an additional 0.5 l. of water, and the final lot of 5 after with 1.0 l. more of water. Preliminary work indicated that an interval of 2 weeks was necessary between spraying and the washing of the plates with water. The total amount of water reaching each plate in all 3 washings was approximately 0.485 inches.

The spore germination tests of the 1940 and 1941 materials as determined by S. E. A. McCallan involved the use of a settling tower (McCallan and Wilcoxon (17)) for spraying glass plates, and the determination of LD50 values (Wilcoxon and McCallan (26)) for *Sclerotina fructicola* on unwashed slides and on those subjected to an inch of laboratory rain delivered through a rose-type nozzle (McCallan and Wilcoxon (16)). From the ratio LD50 weathered slides/LD50 unweathered slides the tenacity index was obtained.

The field spraying was done with a commercial-type orchard sprayer on cherry trees not more than 15 years of age. Single-tree randomized plots replicated at least 6 times were always employed. After certain preliminary work it was found necessary to take approximately 180 leaves per plot for the method of analysis used prior to 1941 and 50 per plot in that year. Sampling in 1940 and 1941 was confined to fruit spur leaves on cherries (Montmorency).

The area of leaves was determined photoelectrically after the method of Frear (4). Since the use of alternating current introduced an error due to fluctuation in the original reading, an apparatus was built in 1940 of the same type but using storage batteries with a current adjustment that always gave the same amount of light. Copper analyses were expressed as milligrams per square meter (both plates and leaves). Leaf-area calculations were based on 2 surfaces per leaf.

Methods of copper analysis on plates and leaves have been described by Frear (5) and involve the use of a photoelectric colorimeter. Prior to 1941 leaves were analyzed for copper by the electrolytical method.

Preliminary studies in 1938 on apple and cherry and, in 1939, on the latter, as well as considerable laboratory work, indicated that certain refinements in technique were necessary for obtaining accurate determinations of retention. Marked differences in retention were found for certain of the copper materials tested, but extreme variability precluded any valid con-

clusions. In view of this, retention studies in the field in 1940 and 1941 were confined to cherry leaves where the copper residue was found to be less variable than the apple fruit and leaf surfaces. Copper analyses were made separately for each of the 6 replicates in order that the data might be subjected to analysis of variance. The spray formula was exactly the same in both laboratory and field.

## RESULTS

The kind and amount of materials used for each treatment in both laboratory and field are given in table 1. The tank-mix copper phosphate used in 1940 was prepared by dissolving 2 lb. of copper sulphate directly into the tank and then adding 2 lb. of trisodium (Treatment 7) or disodium (Treatment 22) phosphate. Three pounds of hydrated lime were added last. Instant Bordeaux mixture was prepared following the procedure described by Schneiderhan (25). Lead arsenate was used at the rate of 2 lb. per 100 in the first 3 sprays in 1940 and in the first 2 in 1941.

The standard 4-spray schedule, consisting of petal-fall, shuck-fall, pink-fruit, and post-harvest applications, was supplied in the field. The first sample was taken in the field the same day that the post-harvest spray was made, and the residue found at this time is referred to as the original deposit. This deposit consisted of a weathered residue remaining from previous applications and the unweathered portion from the spray applied immediately before the sampling. The variation in the amount of copper found at this time as given in table 1 is suggested to be a reflection of the varying retention from the previous sprays as well as of the normal variation in field spraying. The laboratory spraying involved only one application with no lead arsenate since some preliminary work indicated that this material did not significantly affect retention of copper on the plates.

To compare the retention of various materials, calculations of percentage copper remaining were made based on this original residue on the leaves and the unwashed plates in the laboratory. These percentages are given in table 1, together with actual amounts of copper in the non-weathered deposit on the leaves and unwashed plates.

In order to facilitate comparisons of the retention on leaves and on Pyralin plates with that obtained by spore germination tests, indices of retention were calculated from the data in table 1 to give a single figure to express the retention of a given material on either the plates or leaves. The percentage of copper remaining on the leaves at a given sampling date was multiplied by the total rainfall before this sampling and a total obtained by adding these 3 values for each of the treatments. The index was calculated by dividing this total by the sum of the rainfall for the 3 weathering periods multiplied by 100 to obtain a value less than 1. Thus the index for treatment no. 1 in 1940 was obtained as follows:

$$\frac{(100.5 \times 3.7) + (72.9 \times 2.8) + (38.2 \times 4.9)}{(3.7 + 2.8 + 4.9) \times 100} = 0.669$$

TABLE 1.—*Original copper deposits and percentages remaining after weathering of leaves and washing of plates*[a]

| Treatment[b] | Leaves | | | | Plates | | | |
|---|---|---|---|---|---|---|---|---|
| | Sampling | | | | Amt. water sprayed on plates | | | |
| | 1st (orig.) | 2nd | 3rd | 4th | None (orig.) | 0.25 l. | 0.5 l. | 1.0 l. |
| *1940* | *Mg.* | *Per cent* | *Per cent* | *Per cent* | *Mg.* | *Per cent* | *Per cent* | *Per cent* |
| 1. Bord. 2–4–100[c] | 38.7 | 100.5 | 72.9 | 38.2 | 21.8 | 99.8 | 101.5 | 100.1 |
| 7. Tank-mix copper phos. | 40.6 | 91.2 | 67.5 | 38.1 | 20.8 | 101.6 | 101.2 | 100.9 |
| 11. Tenn. "26" 3–3–100 | 36.5 | 66.1 | 45.2 | 16.1 | 35.2 | 85.7 | 63.9 | 51.8 |
| 12. " +1 pt. Orthex | 43.6 | 74.8 | 57.2 | 23.3 | 35.8 | 90.9 | 73.8 | 62.4 |
| 13. " +1 pt. Nufilm | 40.3 | 56.4 | 45.6 | 11.7 | 30.1 | 94.1 | 81.1 | 65.1 |
| 14. " +1 pt. Spralastic | 46.7 | 73.9 | 49.1 | 18.1 | 35.5 | 90.2 | 69.0 | 53.0 |
| 15. " +Summermulsion Spredrite | 45.4 | 79.8 | 60.8 | 23.3 | 35.5 | 98.2 | 83.3 | 70.6 |
| 22. Tank-mix copper phos. | 44.7 | 79.6 | 66.5 | 29.8 | 22.0 | 99.6 | 100.3 | 101.6 |
| Least sig. diff. (19:1) | ..... | 12.1 | 9.9 | 7.0 | ..... | ..... | ..... | ..... |
| *1941* | | | | | | | | |
| 3. Bord. 2–8–100 | 38.4 | 68.1 | 41.1 | 30.0 | 18.0 | 97.1 | 88.4 | 89.9 |
| 4. Cupro-K 3–3–100 | 40.0 | 50.3 | 35.5 | 25.6 | 20.3 | 92.6 | 63.9 | 50.1 |
| 5. " +1 pt. S.E.C. oil | 47.5 | 62.8 | 42.7 | 37.2 | 28.5 | 90.7 | 82.0 | 84.3 |
| 6. Tenn. "26" 3–3–100 | 42.2 | 41.3 | 36.3 | 24.4 | 30.6 | 83.1 | 56.9 | 44.1 |
| 7. " (dolomitic lime) | 45.3 | 43.1 | 29.8 | 25.6 | 33.7 | 65.7 | 51.5 | 40.2 |
| 8. " "34" 2¼–3–100 | 40.9 | 45.4 | 34.2 | 31.5 | 29.2 | 78.8 | 72.3 | 54.6 |
| 9. Copper Hydro "40" 3–3–100 | 53.2 | 38.4 | 28.2 | 30.8 | 33.9 | 77.3 | 52.5 | 35.6 |
| 10. Copper "A" 1½–3–100 | 33.3 | 34.5 | 22.6 | 24.0 | 25.1 | 70.2 | 46.0 | 31.5 |
| 11. Coposil 3½–3–100 | 35.0 | 35.7 | 28.4 | 23.5 | 28.4 | 74.3 | 54.5 | 32.3 |
| 12. Bordow 6–3–100 | 41.7 | 35.3 | 32.1 | 20.6 | 31.9 | 75.6 | 57.5 | 48.7 |
| 15. As 6+1 qt. Spralastic | 53.7 | 43.2 | 34.0 | 24.4 | 35.8 | 54.2 | 51.9 | 37.8 |
| 16. As 6+½ lb. soya flour | 46.7 | 33.5 | 32.7 | 21.9 | 27.8 | 60.3 | 41.2 | 36.5 |
| 18. Copper "A" 1½–8–100 | 33.5 | 34.7 | 29.5 | 25.0 | 28.3 | 55.5 | 37.0 | 23.7 |
| 20. Same as 6 | 48.3 | 35.6 | 25.7 | 22.2 | | (See Treatment No. 6) | | |
| Least sig. diff. (19:1) | ..... | 6.3 | 5.8 | 4.8 | ..... | ..... | ..... | ..... |

[a] Copper expressed as milligrams per square meter in original deposit and as percentage of the original after the three weathering periods in the field and three washings in the laboratory. Percentage values are an average of the percentage remaining in six blocks in the field and of two tests of five plates each in the laboratory.

[b] Treatment numbers omitted were not sampled.

[c] The first figure in all the treatment formulae represents pounds of copper material and the second, pounds of hydrated spray lime (high calcium lime, except where indicated otherwise).

The index for plates was obtained in the same way with the percentage copper remaining on the plates and amounts of water used in the washing process. These indices are given in table 2. The control for 1941 is expressed as the percentage leaves on October 10 with no leaf spot (*Coccomyces hiemalis*) on 4 tagged branches with 50 leaves per tag originally present. Differences in control in 1940 were not significant and these data are not given. Rainfall for the sampling periods in the field is shown in figure 1.

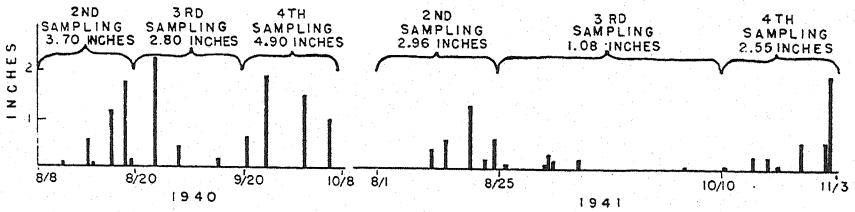



FIG. 1. Sampling dates and distribution of rainfall in 1940 and 1941. Total rainfall for each weathering period also is given.

TABLE 2.—*Summary of data obtained in 1940 and 1941*

| Treatment | Retention index[a] | | Tenacity index | LD50 | Control |
|---|---|---|---|---|---|
| | Plates | Leaves | | | |
| *1940* | | | | | |
| 1. Bord. 2–4–100 | 1.000 | 0.669 | 0.555 | 0.190 | ..... |
| 7. Tank-mix copper phos. | 1.010 | 0.626 | 0.465 | 0.230 | ..... |
| 11. Tenn. "26" 3–3–100 | 0.605 | 0.395 | 0.190 | 0.350 | ..... |
| 12. " +1 pt. Orthex | 0.698 | 0.483 | 0.235 | 0.360 | ..... |
| 13. " +1 pt. Nufilm | 0.738 | 0.345 | 0.170 | 0.330 | ..... |
| 14. " +1 pt. Spralastic | 0.629 | 0.438 | 0.085 | 0.300 | ..... |
| 15. " +Summermulsion Spredrite | 0.782 | 0.508 | 0.070 | 0.240 | ..... |
| 22. Tank-mix copper phos. | 1.000 | 0.550 | 0.410 | 0.210 | ..... |
| *1941* | | | | | |
| 3. Bord. 2–8–100 | 0.905 | 0.489 | 0.570 | 0.150 | 64.7 |
| 4. Cupro-K 3–3–100 | 0.602 | 0.383 | 0.345 | 0.440 | 20.2 |
| 5. " +1 pt. S.E.C. oil | 0.845 | 0.495 | 0.240 | 0.590 | 27.7 |
| 6. Tenn. "26" 3–3–100 | 0.531 | 0.339 | 0.150 | 0.460 | 45.4 |
| 7. " (dolomitic lime) | 0.471 | 0.341 | 0.135 | 0.320 | 46.7 |
| 8. " "34" 2¼–3–100 | 0.631 | 0.382 | 0.310 | 0.360 | 54.2 |
| 9. Copper Hydro "40" 3–3–100 | 0.464 | 0.337 | 0.305 | 0.590 | 42.1 |
| 10. Copper "A" 1½–3–100 | 0.412 | 0.285 | 0.185 | 0.350 | 41.0 |
| 11. Coposil 3½–3–100 | 0.447 | 0.298 | 0.140 | 0.370 | 34.3 |
| 12. Bordow 6–3–100 | 0.548 | 0.291 | 0.255 | 0.220 | 61.7 |
| 15. As 6+1 qt. Spralastic | 0.442 | 0.344 | 0.185 | 0.440 | 41.4 |
| 16. As 6+½ lb. soya flour | 0.413 | 0.289 | 0.330 | 0.470 | 43.4 |
| 18. Copper "A" 1½–8–100 | 0.321 | 0.301 | 0.085 | 0.300 | 32.0 |
| 20. Same as 6 | ..... | 0.288 | ..... | ..... | ..... |

[a] Retention index was determined on Pyralin plates in laboratory and cherry leaves in the field. Control is expressed as percentage disease-free leaves. Tenacity index and LD50 were determined by S. E. A. McCallan.

An inspection of table 2 indicates a good correlation between certain of the methods. It is to be noted that in both years the laboratory washing method used on Pyralin plates failed to cause any loss of the Bordeaux mix-

ture residue, although the technique used in the spore-germination tests on glass plates did. The same was true for the 2 tank-mix copper phosphate treatments in 1940. These materials also showed a higher retention on the leaves than any of the Tennessee "26" formulae in that year. Summermulsion and Spredrite, Spralastic, and Orthex all acted as stickers in increasing the retention of Tennessee "26" on Pyralin plates and leaves. Nufilm did not increase retention on the leaves nor on the glass plates in the spore-germination tests. Tennessee "34" was higher in retention than Tennessee "26" on all three surfaces and also in control. Copper "A" was low in all the tests, as well as in control, and the additional lime in treatment 18 appeared to have no effect. Tennessee "34" was better than Copper Hydro "40" in all 3 tests. Dolomitic lime with Tennessee "26" did not affect retention or control as compared with treatment 6 using the regular high calcium spray lime. Cottonseed oil emulsion (S.E.C. oil) increased retention of Cupro-K, as well as control. Copper "A" and Coposil were low in retention in all 3 tests, but Bordow was low on the leaves.

In order to compare more accurately the correlations of the data given in table 2, correlation coefficients were calculated (Table 3). There is evi-

TABLE 3.—*Correlation of retention indices on leaves and plates, tenacity indices, LD50 and control as given in table 2*[a]

| Factors correlated | Year | Correlation coefficient |
|---|---|---|
| Retention indices on Pyralin plates and leaves | 1940 | 0.845 |
| | 1941 | 0.930** |
| Retention index on leaves, and tenacity coefficient | 1940 | 0.809** |
| | 1941 | 0.584* |
| Retention index on Pyralin plates, and tenacity coefficient | 1940 | 0.856** |
| | 1941 | 0.679** |
| Control and retention index on leaves | 1941 | 0.034 |
| Control and retention index on Pyralin plates | 1941 | 0.247 |
| Control and tenacity coefficient | 1941 | 0.413 |
| Control and LD50[b] | 1941 | −0.614* |
| Control, retention index on leaves, and LD50 | 1941 | 0.617 |
| Control, retention index on plates, and LD50 | 1941 | 0.641 |
| Control, tenacity coefficient, and LD50 | 1941 | 0.679* |

* Significant at odds 19: 1.
** Significant at odds 99: 1.
[a] Plot 20, in 1941, omitted.
[b] Negative coefficient, because low LD50 indicates high toxicity.

dence from these data of a high correlation between retention on Pyralin plates and on leaves, as determined by the chemical-analysis procedure. The tenacity index also showed a significant correlation with the leaf retention, although, in 1941, the coefficient was not so high as that obtained by the direct-analysis method. The tenacity index was slightly better correlated with control than the direct analysis determination on plates although neither was significant. A low correlation was also found between retention on leaves and control.

The LD50 values for the treatments used in 1941 did show a significant correlation with the control data, suggesting that the toxicity of the material

rather than retention was the important factor influencing the protective value in the field. When the additional factor of retention was correlated with control and LD50 by means of multiple correlation, a slight increase in the coefficient was obtained. The highest multiple correlation coefficient obtained was between control, tenacity index, and LD50, and is high enough to be significant with the lower number of degrees of freedom (n – 3) required for 3 factors.

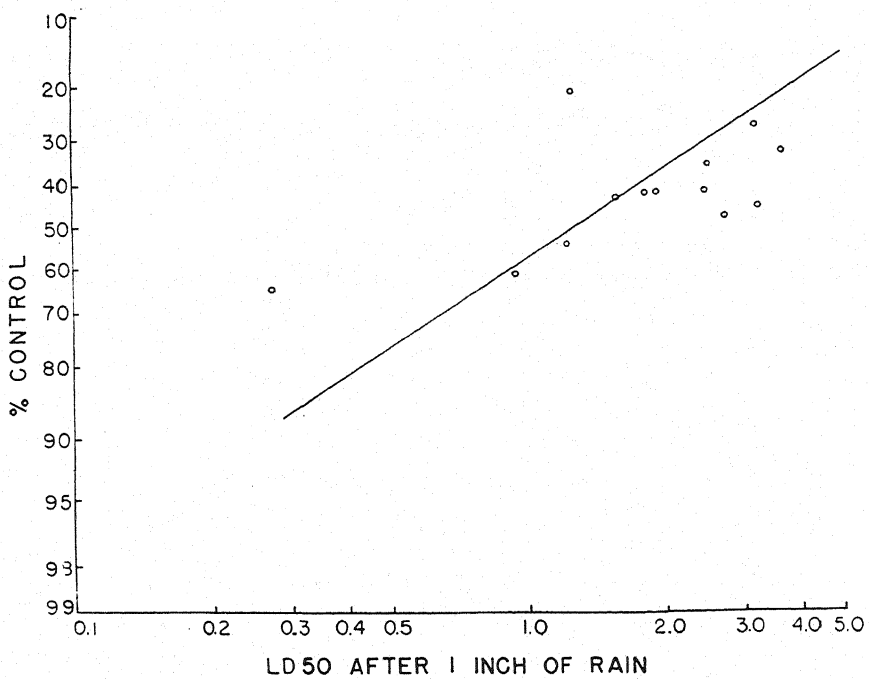



FIG. 2. LD50 values after rain plotted against control (percentage disease-free leaves) on logarithmic probability paper.

The relation between the LD50 after a rain test (LD50/tenacity index) and control expressed as disease-free leaves is shown in figure 2 on logarithmic probability paper. A distinct trend is noted excepting for two points. One of these points is Bordeaux mixture and the other is Cupro-K. The latter material has been found in laboratory tests to be much more soluble than the others and would probably explain its high toxicity. By ignoring this point (LD50 1.26) can be stated that this indicates that percentage control is normally distributed against the reciprocal of the logarithm of the LD50 after the rain test. Such a relationship emphasizes the need for LD50 values in the laboratory toxicity tests after being subjected to a laboratory rain.

## DISCUSSION

The direct chemical analysis was used in the retention studies on Pyralin plates in the laboratory since it was assumed that this would make the deter-

minations more comparable with that obtained for the leaves in the field when this was the only practicable method to use. The high correlation between the retention on leaves and Pyralin plates indicates the validity of this assumption. However, the fact that there was so little correlation between retention of actual copper and control indicates that some other more important factor was operating to limit the protective value in the field. Toxicity of the material must have been this factor, since the LD50 values gave such a high correlation with control. Retention was involved, but was of much less importance than toxicity.

Although the 3 methods of evaluating retention correlated very well, certain exceptions were found for which no satisfactory explanation seems available. There are, of course, many factors that are different in the laboratory and field tests. Martin (20), for example, has pointed out that in field spraying many leaves are sprayed beyond the point of run-off, while this is not done in ordinary laboratory tests. Worthley and Frear (27) suggested that the application of more than one spray to the leaves probably caused some discrepancy between results on leaves and on Pyralin plates in a study of deposition and retention of lead arsenate. However, in some preliminary work with the copper fungicides used in this study, more variation as indicated by a much higher standard error for the original residue was found where only the post-harvest application of a copper material had been made than where 3 copper sprays had preceded this.

The fact that a cherry leaf falls as soon as it becomes heavily infected with leaf spot or injured with spray, tends to cause an increase in the percentage of leaves with a high residue on treatments giving inferior control and a lower residue on treatments that cause excessive injury. No injury was apparent in the 1940 and 1941 tests.

Difference in the chemical and physical nature between leaves and the surfaces used in the laboratory must be considered. The weathering process in the laboratory consists of washing with water alone; yet, in the field, mechanical abrasion (7), alternate wetting and drying, fluctuations in temperature, and sunlight, probably, also are involved. In fact, so many differences exist that it seems especially significant to have data that indicate high correlation between the two types of test.

The correlation between retention as determined by direct chemical analysis and, as determined by the spore germination technique, seems significant in view of the statement by Heuberger (12) that the latter method determines both the quantity and fungicidal value of the residue remaining after weathering. The correlation of the two methods with retention on leaves was good in 1940; yet the spore germination test did not correlate so well in 1941 with the actual quantitative measurements on either leaves or plates. Horsfall (13) also found that 2 spray deposits having the same quantity of copper may not inhibit spore germination to the same extent. Martin (21) has noted certain changes in the composition of Bordeaux mixture upon drying, which would be expected to change the fungicidal

value of the residue and also would interfere with a quantitative measurement of the residue by a method depending on spore-germination tests without necessarily affecting the actual amount of copper retained.

An indication of the error involved in field determinations of retention can be found in table 2 where treatments 6 and 20 are the same in 1941, yet the retention index on the leaves differs considerably. This emphasizes the importance of a statistical approach to the interpretation of field data.

A comparison of data on the commercial compounds used in this study with those reported by other workers is difficult, since there is no certainty that the composition is the same from year to year, even though the trade name remains unchanged. Heuberger (12) found that soya flour did not increase retention, which agrees with the results obtained with Tennessee "26" in 1941. Hartmann (10) reported Cupro-K and Compound (Copper) "A" were lower in retention than Bordow, and that these 3 were lower than Bordeaux mixture and Copper Hydro "40," which is in accord with the 1941 results reported here.

The host plant, according to Horsfall (13) does not alter the ranking of materials studied by laboratory methods. Martin (21), on the other hand, has pointed out the importance of the host plant in changing the protective value of a fungicide. However, high correlations found in this study indicate that the laboratory methods are fairly accurate in predicting the behavior of materials in the field, although it is evident that some few may not perform as expected.

## SUMMARY

The retention of several protective copper fungicides was determined in the laboratory by the direct chemical analyses of Pyralin plates sprayed under standard conditions. This showed a very high correlation with direct analytical determination of retention of the same materials on cherry leaves in 2 different years. These 2 methods also correlated very well with a determination of retention by the spore-germination technique.

Bordeaux mixture and 2 formulae of tank-mix copper phosphate showed a higher retention than any of the commercial forms of copper materials. Summermulsion and Spredrite, Spralastic, and Orthex all increased the retention of Tennessee "26" on leaves, while soya flour and Nufilm reduced it. Cottonseed oil emulsion increased the retention of Cupro-K. Tennessee "34" was retained better than Tennessee "26" or Copper Hydro "40." The retention of Tennessee "26" was the same with dolomitic lime as with regular high calcium lime. Coposil, Copper "A" and Bordow were low in retention on foliage.

Very little correlation was found between control and retention of copper on cherry leaves in the field and retention on Pyralin plates in the laboratory or the tenacity index. There was a significant correlation between control and toxicity expressed as the LD50. A significant correlation between control, tenacity index, and LD50 also was found. The percentage control

expressed as disease-free leaves was found to be normally distributed against the logarithm of the LD50 values after the laboratory rain.

It is concluded that the laboratory methods of determining retention predicted fairly accurately the retention of the same materials in the field, but that toxicity was a much more important factor than retention in predicting the protective value of the fungicides.

DEPARTMENT OF BOTANY,
PENNSYLVANIA STATE COLLEGE,
STATE COLLEGE, PA.

LTERATURE CITED

1. Anonymous. Tentative definitions of fungicide terms. Am. Phytopath. Soc. Committee on Standardization of Fungicidal Tests (Mimeo.). Jan., 1942.
2. DAINES, R. H. Two years' experiments in the control of cherry leaf spot (*Coccomyces hiemalis*). (Abstract) Phytopath. 29: 5–6. 1939.
3. FAJANS, E., and H. MARTIN. The incorporation of direct with protective insecticides and fungicides. II. The effects of spray supplements on the retention and tenacity of protective deposits. Jour. Pomol. and Hort. Sci. 15: 1–24. 1937.
4. FREAR, D. E. H. Photoelectric apparatus for measuring leaf areas. Plant Physiol. 10: 569–574. 1935.
5. ————. Determination of small amounts of copper in spray residues. Ind. Eng. Chem. 11: 494–495. 1939.
6. GIRARD, AIMÉ. Recherches sur l'adhérence aux feuilles des plantes, et notamment aux feuilles de la pomme de terre, des composés cuivriques destinés à combattre leurs maladies. Compt. Rend. Acad. Sci. 114: 234–236. 1892.
7. GREEN, E. L., and M. C. GOLDSWORTHY. The copper content of residues from sprays containing adjuvants. Phytopath. 27: 957–970. 1937.
8. GUILLON, G. M., and G. GOUIRAND. Sur l'adhérence des bouillies cupriques ultilisées pour combattre les maladies cryptogramiques de la vigne. Compt. Rend. Acad. Sci. 127: 254–256; 423–424. 1898.
9. HAMILTON, J. M. Studies on apple scab and spray materials for its control in the Hudson Valley. N. Y. State (Geneva) Agr. Exp. Stat. Tech. Bull. 227. 1935.
10. HARTMANN, H. T. Tests with new copper fungicides with special reference to injury, tenacity to foliage, and dwarfing effect. Proc. Am. Soc. Hort. Sci. 38: 148–152. 1941.
11. HEUBERGER, J. W. A laboratory biological assay of tenacity of fungicides. Phytopath. 30: 840–847. 1940.
12. ————. Tenacity of protective fungicides. Chron. Bot. 7: 9–10. 1942.
13. HORSFALL, J. G. Biological assay of protective fungicides. Chron. Bot. 6: 292–294. 1941.
14. KADOW, K. H., S. L. HOPPERSTEAD, and M. W. GOODWIN. Will insoluble copper sprays control bitter rot under conditions favorable to the disease? Trans. Peninsula Hort. Soc. 32(1938): 99–102. 1939.
15. KEHLHOFER, W. Ueber die Ausführung und die Ergebnisse von Hartfertigkeitsversuchen Kupferhaltiger Bekämpfungsmittel gegen die Peronospora. Zeitschrift f. Pflanzenkr. 17: 1–12. 1907.
16. MCCALLAN, S. E. A., and F. WILCOXON. Laboratory comparisons of copper fungicides. Contrib. Boyce Thompson Inst. 9: 249–263. 1938.
17. ————, and ————. An analysis of factors causing variation in spore germination tests of fungicides. II. Methods of spraying. Contrib. Boyce Thompson Inst. 11: 309–324. 1940.
18. MAGIE, R. O., and J. G. HORSFALL. Relative adherence of cuprous oxide and other copper fungicides. (Abstract) Phytopath. 26: 100–101. 1936.
19. MARSAIS, P., and L. SEGAL. Contribution a l'étude de l'action anticryptogamique du cuivre. Rev. Vitic. 88: 285–287. 1938.
20. MARTIN, H. Studies upon the copper fungicides. I. The interaction of copper sulphate with calcium hydroxide. Ann. Appl. Biol. 19: 98–120. 1932.
21. ————. The laboratory examination of fungicidal dusts and sprays. Ann. App. Biol. 19: 263–271. 1932.
22. ————. Studies upon the copper fungicides. II. Some modifications of Bordeaux mixture designed to overcome practical difficulties in its application. Ann. App. Biol. 20: 342–363. 1933.

23. MILLARDET, A., and M. E. DAVID. Résultats de divers procédés de traitement sur le développement du mildiou. Jour. Agr. Pratique. 50(2): 764–770. 1886.
24. PERRAUD, J. Recherches sur quelques moyens permettant d'augmenter l'adhérence des bouillies cupriques. Comp. Rend. Acad. Sci. 127: 876–879. 1898.
25. SCHNEIDERHAN, F. J. Instant Bordeaux. W. Va. Agr. Exp. Stat. Circ. 60. 1932.
26. WILCOXON, F., and S. E. A. MCCALLAN. Theoretical principles underlying laboratory toxicity tests of fungicides. Contrib. Boyce Thompson Inst. 10: 329–338. 1939.
27. WORTHLEY, H. N., and D. E. H. FREAR. Deposition of lead from lead arsenate spray mixtures, and its retention on Pyralin plates, apple leaves, and fruits. Jour. Econ. Ent. 35: 205–210. 1942.
28. YOUNG, H. C., and J. R. BECKENBACH. Spreader materials for insoluble copper sprays. Phytopath. 26: 450–455. 1936.

# STUDIES OF THE SUSCEPTIBILITY OF FORAGE GRASSES TO CEREAL SMUT FUNGI. IV. CROSS-INOCULATION EXPERIMENTS WITH UROCYSTIS TRITICI, U. OCCULTA, AND U. AGROPYRI[1]

GEORGE W. FISCHER AND C. S. HOLTON[2]

(Accepted for publication February 19, 1943)

## INTRODUCTION

Plant pathologists and mycologists have long noted the morphological similarity within 3 species of *Urocystis*, causing flag smut of grasses, wheat (*Triticum aestivum* L.), and rye (*Secale cereale* L.), namely, *Urocystis agropyri* (Preuss) Schroet., *U. tritici* Koern., and *U. occulta* (Wallr.) Rab., respectively. Until recently, however, no similarity in host relationships had been demonstrated. McAlpine (12) tried repeatedly to cross-infect wheat and rye with their respective flag smuts, and, being unsuccessful, concluded that the two species (*U. tritici* and *U. occulta*) are distinct. Griffiths (5) inoculated seed of redtop (*Agrostis alba* L.), timothy (*Phleum pratense* L.), rye, and wheat with flag smut from wheat and rye. The inoculum from wheat infected only wheat, and that from rye infected only rye. Jarrett (10) was unsuccessful in repeated attempts to extend the host range of *U. tritici* to other cereals and to grasses. and concluded that the flag smut of wheat is strictly limited to wheat. Recently, however, there was reported (3) the results of the first partially successful attempts at cross inoculation within these 3 species.

All 3 species of *Urocystis* mentioned above occur in the United States. Flag smut of grasses and flag smut of rye (also improperly called stem smut)[3] have long been known in this country, but flag smut of wheat is of comparatively recent appearance, having been discovered in the middle west first by S. M. Zeller in 1918, and then by J. G. Dickson in 1919 according to Humphrey and Johnson (9), and to Tisdale *et al.* (16). In 1940 it was reported from central Washington by Heald and Holton (6).

The origin of flag smut of wheat in the United States has been a much-discussed matter. Brittlebank (1), according to Tisdale *et al.* (15), was of the opinion that the disease probably had been introduced with the 5,500,000 bushels of wheat exported to the United States from Australia in 1918. This

[1] Cooperative investigations of the Divisions of Forage Crops and Diseases, and Cereal Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, United States Department of Agriculture, with the Washington State Agricultural Experiment Station, Pullman, Washington, and the Soil Conservation Service, United States Department of Agriculture. Published with the approval of the Experiment Station Director as Scientific Paper No. 539.

[2] Associate Pathologist, Division of Forage Crops and Diseases, and Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, United States Department of Agriculture.

[3] The designation of "stem smut" should be reserved for those smuts that involve the stem only, such as is typified by *Ustilago hypodytes* (Sch.) Fries. Flag smut of rye sporulates chiefly in the leaves and leave sheaths, and, even though the culm and inflorescence may be involved, the typical striped or "flag" aspect is obvious.

opinion prevailed until it was pointed out by Tisdale *et al.* (16), in 1927, that actually the first collection of flag smut of wheat in this country was made in 1918. Tisdale *et al.* (16) explained that the disease had to be present in the middle west at sowing time in 1917 in order to infect the crop of 1918, but added that this did not preclude the possibility of flag smut having been introduced into this country along with earlier shipments of wheat or other products from Australia or some other country.

In 1938 the senior writer found squirrel-tail grass (*Sitanion jubatum* L.), seriously infested (50 per cent) with flag smut (*Urocystis agropyri*) in Klickitat County, Washington. No particular attention was paid to this incidence of flag smut in squirrel-tail grass until its discovery in wheat 2 years later in the same locality. This led to some conjecturing as to the possible relationship that might exist between the flag smut of squirrel-tail grass and that of wheat. Flag smut of grasses has been long prevalent throughout the country on a wide variety of grasses (2, 17), and since no adequate explanation has been provided as to the source of the recent outbreak of the wheat flag smut in Washington, as well as in the Middle West in 1918 and 1919, the question arose as to whether these outbreaks represented strains of the similar smut of grasses that were capable of attacking wheat. The morphologically identical flag smut of grasses occurs in the Northwest on many species of *Agropyron, Bromus, Elymus, Festuca, Hordeum, Melica, Poa,* and *Sitanion,* and if the host range over the United States as a whole be considered, other genera could be added. This being the case, it seems reasonable that cultivated wheat might also be included, especially if common susceptibility could be demonstrated. Evidence of this could be obtained only from cross-inoculation experiments, the results of which are presented in this paper, together with a discussion of their bearing on the whole problem of flag smut in the United States, especially with regard to host relationships.

## METHODS AND MATERIALS

Inoculation of grasses was effected by processing the seed in an aqueous suspension of smut spores, under partial vacuum. Wheat and rye were inoculated by dusting the seed with spores. All tests with wheat and rye were run in the greenhouse, as were most of the grasses, although some were planted in the field. These latter were started in the greenhouse in wood veneer plant bands and later transplanted as seedlings to the field. Since spores of *Urocystis agropyri* germinate more slowly than spores of *U. tritici* and *U. occulta,* they were soaked in tap water in the refrigerator at 2–5° C. for several weeks before being used. No pre-soaking was necessary for *U. tritici* and *U. occulta.*

Inocula of *Urocystis agropyri* were obtained from collections made during the past several years. The infected plant parts were macerated in water and the spores thus freed were recovered by straining through cheesecloth. Two collections of *U. tritici* were used in these studies, one of which

came from Leavenworth, Kansas,[4] and the other from Goldendale, Washington. The inoculum of *U. occulta* was obtained from University Farm, St. Paul, Minnesota.[5]

## RESULTS

Some greenhouse experiments of a very preliminary nature were carried out in 1940. One or more accessions of *Agropyron, Elymus, Hordeum,* and *Sitanion* spp. were inoculated with 3 collections of *Urocystis agropyri* and 1 of *U. tritici. Agropyron inerme* (Scribn. and Smith) Rydb., alone became infected with *U. tritici,* while *A. trachycaulum* (Link) Malte, *A. spicatum* (Pursh) Scribn. and Smith, *A. inerme, Elymus glaucus* Buckl., *E. canadensis* L., *Sitanion jubatum,* and *S. hystrix* (Nutt.) J. G. Smith were infected with *U. agropyri.*

## GREENHOUSE-FIELD PLANTINGS

Seed of many species of *Agropyron, Bromus, Elymus, Festuca, Hordeum, Lolium,* and *Sitanion* was inoculated with *Urocystis agropyri* (from *Hordeum nodosum,* Corvallis, Oregon), two collections of *U. tritici* and one of *U. occulta.* The inoculated seed was planted in the greenhouse in late winter (1941) and the seedlings were transplanted to the field early the following spring.

TABLE 1.—*Results of inoculations of grasses with flag smut of wheat, flag smut of rye, and flag smut of grasses (Urocystis tritici, U. occulta, and U. agropyri respectively)*[a]

| Species | Acc. No.[b] | Source and identity of inoculum | | | |
|---|---|---|---|---|---|
| | | *Urocystis agropyri* | *Urocystis tritici* | | *Urocystis occulta* |
| | | Oregon | Washington | Kansas | Minnesota |
| *Agropyron caninum* | F 138 | --[c] | − | + | − |
| *Agropyron caninum* | F 139 | + | + | + | + |
| *Agropyron inerme* | W 2522 | − | − | −− | + |
| *Agropyron repens* | F 140 | −− | + | + | − |
| *Agropyron semicostatum* | W 2903 | −− | + | + | − |
| *Agropyron spicatum* | W 736 | −− | + | − | − |
| *Agropyron spicatum* | W 739 | −− | − | + | − |
| *Elymus canadensis* | W 786 | −− | − | − | + |
| *Elymus canadensis* | W 788 | + | − | −− | + |
| *Elymus canadensis* | W 2389 | + | − | −− | + |
| *Elymus canadensis* | W 3549 | + | − | −− | − |
| *Elymus canadensis* | W 4669 | + | − | −− | − |
| *Elymus glaucus* | W 1753 | −− | + | − | − |
| *Elymus glaucus* | W 2662 | − | + | −− | − |
| *Elymus triticoides* | W 2741 | −− | + | − | − |

[a] Many species of *Bromus, Festuca, Hordeum, Lolium,* and *Sitanion* were included also but did not become infected. For the sake of brevity these are not listed.

[b] "F" numbers are those of the senior writer. "W" numbers are those of the Soil Conservation Service, United States Department of Agriculture, Pullman Nursery Unit.

[c] −− = not inoculated.
+ = typical flag-smut symptoms developed.
− = no signs of infection.

[4] Supplied by A. G. Johnson, Bureau of Plant Industry.
[5] Supplied by J. J. Christensen.

The results of this experiment are presented in table 1, where only those species are listed that proved susceptible to one or more of the flag smut collections. The perennial nature of this infection was shown by the fact that plants observed to be infected in 1941 showed flag smut again in 1942. The percentages of infection were quite variable, ranging from a trace to nearly 50 per cent (*U. occulta* on *E. canadensis*).

## EXPERIMENTS IN THE GREENHOUSE

Where there was sufficient spore material, inoculum was prepared from the above infected grasses (Table 1) infected with flag smuts of wheat, rye,

TABLE 2.—*Results of inoculations of wheat, rye, and grasses with flag smut (Urocystis agropyri, U. tritici and U. occulta) taken from grasses artificially infected with flag smut*

| Smut | Original source | Immediate source | Grass or cereal inoculated | Per cent[b] smut |
|---|---|---|---|---|
| *U. agropyri* | *Hordeum nodosum*, Oregon | *Elymus canadensis* W 4669 | *Elymus canadensis* W 4669 | 3.4 |
| *U. agropyri* | *Hordeum nodosum*, Oregon | *Elymus canadensis* W 2389 | *Elymus canadensis* W 2389 | 8.5 |
| *U. agropyri* | *Hordeum nodosum*, Oregon | *Elymus canadensis* W 3549 | Wheat[a] | 0.0 |
| *U. agropyri* | *Hordeum nodosum*, Oregon | *Elymus canadensis* W 3549 | Rye (Prolific) | 0.0 |
| *U. tritici* | Wheat, Washington | *E. glaucus* W 1753 | *E. glaucus* W 1753 | 34.7 |
| *U. tritici* | Wheat, Washington | *E. glaucus* W 1753 | Wheat | 33.7 |
| *U. tritici* | Wheat, Washington | *A. caninum* F 139 | *A. caninum* F 139 | 0.0 |
| *U. tritici* | Wheat, Washington | *A. caninum* F 139 | Wheat | 20.4 |
| *U. tritici* | Wheat, Washington | *E. triticoides* W 2741 | *E. triticoides* W 2741 | 0.0 |
| *U. tritici* | Wheat, Washington | *E. triticoides* W 2741 | Wheat | 39.4 |
| *U. tritici* | Wheat, Washington | *A. repens* F 140 | *A. repens* F 140 | 1.4 |
| *U. tritici* | Wheat, Washington | *A. repens* F 140 | Wheat | 11.4 |
| *U. tritici* | Wheat, Washington | *A. semicostatum* W 2903 | *A. semicostatum* W 2903 | 5.3 |
| *U. tritici* | Wheat, Washington | *A. semicostatum* W 2903 | Wheat | 14.2 |
| *U. tritici* | Wheat, Kansas | *A. caninum* F 138 | *A. caninum* F 138 | 13.7 |
| *U. tritici* | Wheat, Kansas | *A. caninum* F 138 | Wheat | 20.8 |
| *U. tritici* | Wheat, Kansas | *A. spicatum* W 739 | *A. spicatum* W 739 | 5.0 |
| *U. tritici* | Wheat, Kansas | *A. spicatum* W 739 | Wheat | 26.1 |
| *U. tritici* | Wheat, Kansas | *A. repens* F 140 | *A. repens* F 140 | 3.7 |
| *U. tritici* | Wheat, Kansas | *A. repens* F 140 | Wheat | 12.0 |
| *U. occulta* | Rye, Minnesota | *E. canadensis* W 788 | *E. canadensis* W 788 | 4.3 |
| *U. occulta* | Rye, Minnesota | *E. canadensis* W 788 | Rye (Dakold) | 32.8 |
| *U. occulta* | Rye, Minnesota | *E. canadensis* W 2389 | *E. canadensis* W 2389 | 14.2 |
| *U. occulta* | Rye, Minnesota | *E. canadensis* W 2389 | Rye (Dakold) | 35.4 |
| *U. occulta* | Rye, Minnesota | *A. caninum* F 139 | *A. caninum* F 139 | 0.0 |
| *U. occulta* | Rye, Minnesota | *A. caninum* F 139 | Rye (Prolific) | 8.3 |

[a] All wheat listed in this column is Kanred × Hard Federation (C.I. 10092).

[b] Infection percentages based on a range of from 7–215 plants per row, with an average of at least 90.

and grasses, and used to reinoculate their natural hosts. All cereal and grass seed thus inoculated was sown in rows in the greenhouse October 13, 1941. The first symptoms on both grasses and cereals were observed November 29; and on December 26 notes were taken. The results of these inoculations are presented in table 2.

The results (Table 2) fully substantiate those of the field sowings in which several species of grasses were infected with *Urocystis tritici* and *U. occulta.* In every case, excepting check rows, infection was obtained on rye inoculated with *U. occulta* from the artificially infected grasses, and on wheat with *U. tritici* from artificially infected grasses. The fact that the infection percentages on the cereals were generally so much higher than on the grasses leaves little room for any doubt that the apparent infection of the grasses with the cereal flag smut is valid.

Considering the foregoing results of the preliminary inoculations of grasses with flag smut, it seemed desirable to follow up with a more exhaustive test, using several recent collections of *Urocystis agropyri* in addition to *U. tritici* and *U. occulta.* Following the same technique already outlined, seed of the following grass species was inoculated with 4 collections of *U. agropyri,* 2 of *U. tritici,* and 1 of *U. occulta: Agropyron cristatum* (L.) Gaert., *A. dasystachyum* (Hook.) Scribn., *A. desertorum* (Fisch.) Schult., *A. elmeri* Scribn., *A. elongatum* (Host.) Beauv., *A. inerme, A. repens, A. semicostatum, A. sibiricum* (Willd.) Beauv., *A. smithii* Rydb., *A. spicatum, A. subsecundum* (Link) Hitchc., *A. trachycaulum, A. trichophorum* (Link) Richt.; *Elymus canadensis, E. glaucus, E. sibiricus* L., *E. triticoides; Hordeum brevisubulatum* (Trin.) Link, *H. bulbosum* L., *H. jubatum* L., *H. jubatum* var. *caespitosum* (Scribn.) Hitchc., *Poa ampla* Merr., *P. nevadensis* Vasey, *P. pratensis* L., *P. secunda* Presl.; *Sitanion jubatum.* In addition to the above grasses the following cereals were included: Hard Federation wheat (C.I. 4733), Kanred × Hard Federation wheat (C.I. 10092), Prolific rye, Dakold rye, and Rosen rye × *Secale montanum* Guss.[6] The inoculated seed was planted in rows in greenhouse benches, January 1, 1942. On February 3 the first symptoms were noticed in several of the grasses. It was observed at that time, and has been noticed repeatedly since, that flag smut apparently does not appear earlier than on the third seedling leaf. Data were taken beginning March 14, 1942.

The positive results of the above inoculations of grasses and cereals with seven flag smut collections are given in table 3. The grass-host range of both *Urocystis tritici* and *U. occulta* is extended by the above experiment. New hosts for the wheat flag smut are *Agropyron dasystachyum, A. desertorum, A. trachycaulum, Elymus canadensis,* and *Hordeum jubatum* var. *caespitosum. Elymus triticoides* is a new host for *U. occulta.* These results present the first published experimental evidence of the existence of physiologic specialization in the flag smut of grasses, *Urocystis agropyri.* Collections C–I, C–H, and C–F, from *Agropyron trachycaulum, Hordeum jubatum,* and

[6] Cross by D. C. Smith, formerly of the Bureau of Plant Industry.

TABLE 3.—*Positive results of inoculation of species of Agropyron, Elymus, Hordeum, Poa and Sitanion spp., and wheat and rye varieties with 7 Collections of flag smut (Urocystis agropyri, U. occulta and U. tritici) in the Greenhouse 1942*[a]

| Hosts | Flag smut collections[b] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Urocystis agropyri* | | | | | | | | *Urocystis occulta* | | *Urocystis tritici* | | | |
| | C–I | | C–H | | C–G | | C–F | | C–E | | C–D | | C–$D_1$ | |
| | Plants | Smut | Plants | Smut | Plants | Smut | Plants | Smut | Plants | Smut | Plants | Smut | Plants | Smut |
| *Grasses:* | *No.* | *Per cent* | *No.* | *Per cent* | *No.* | *Per cent* | *No.* | *Per cent* | *No.* | *Per cent* | *No.* | *Per cent* | *No.* | *Per cent* |
| *Agropyron dasystachyum* W 4702[c] | 45 | 17.7 | 49 | 2.0 | 59 | 0.0 | 34 | 0.0 | 21 | 0.0 | 43 | 0.0 | 63 | 11.1 |
| *Agropyron dasystachyum* W 7801 | 81 | 9.8 | 69 | 2.8 | 115 | 0.0 | 24 | 0.0 | 48 | 0.0 | 29 | 0.0 | 102 | 0.9 |
| *Agropyron desertorum* W 11 | 186 | 0.0 | 65 | 0.0 | 97 | 0.0 | 26 | 0.0 | 35 | 0.0 | 16 | 0.0 | 74 | 1.3 |
| *Agropyron elongatum* W 2326 | 101 | 0.9 | 90 | 0.0 | 130 | 0.0 | 62 | 0.0 | 98 | 0.0 | 92 | 0.0 | 94 | 0.0 |
| *Agropyron inerme* W 2522 | 107 | 4.6 | 81 | 0.0 | 135 | 0.0 | 26 | 0.0 | 36 | 0.0 | 31 | 0.0 | 47 | 0.0 |
| *Agropyron inerme* W 2617 | 95 | 6.2 | 72 | 0.0 | 204 | 0.0 | 28 | 0.0 | 19 | 0.0 | 29 | 0.0 | 44 | 2.2 |
| *Agropyron repens* F 140 | 67 | 0.0 | 57 | 0.0 | 72 | 0.0 | 56 | 0.0 | 39 | 0.0 | 31 | 0.0 | 99 | 3.0 |
| *Agropyron semicostatum* W 2903 | 52 | 3.8 | 50 | 0.0 | 74 | 0.0 | 27 | 3.7 | 27 | 0.0 | 51 | 0.0 | 111 | 0.0 |
| *Agropyron spicatum* W 736 | 18 | 22.2 | 28 | 0.0 | 41 | 0.0 | 12 | 0.0 | 9 | 0.0 | 17 | 0.0 | 34 | 0.0 |
| *Agropyron spicatum* W 739 | 30 | 3.3 | 32 | 0.0 | 18 | 0.0 | 3 | 0.0 | 4 | 0.0 | 8 | 0.0 | 26 | 0.0 |
| *Agropyron subsecundum* F 13 | 71 | 5.6 | 83 | 0.0 | 87 | 0.0 | 50 | 0.0 | 58 | 0.0 | 66 | 0.0 | 123 | 0.0 |
| *Agropyron trachycaulum* F 67 | 28 | 0.0 | 22 | 0.0 | 83 | 0.0 | 70 | 0.0 | 46 | 0.0 | 75 | 0.0 | 128 | 0.7 |
| *Elymus canadensis* W 2389 | 75 | 13.3 | 66 | 0.0 | 69 | 0.0 | 67 | 13.4 | 41 | 17.0 | 70 | 0.0 | 66 | 9.0 |
| *Elymus canadensis* W 788 | 33 | 51.5 | 72 | 0.0 | 91 | 0.0 | 86 | 17.4 | 60 | 13.3 | 86 | 0.0 | 74 | 2.7 |
| *Elymus glaucus* W 1753 | 36 | 11.1 | 45 | 0.0 | 58 | 0.0 | 33 | 0.0 | 15 | 0.0 | 40 | 10.0 | 48 | 25.0 |
| *Elymus glaucus* W 2662 | 35 | 0.0 | 80 | 0.0 | 60 | 0.0 | 52 | 0.0 | 47 | 0.0 | 70 | 0.0 | 78 | 5.1 |
| *Elymus sibiricus* W 225 | 54 | 1.8 | 25 | 0.0 | 55 | 0.0 | 53 | 0.0 | 39 | 0.0 | 46 | 0.0 | 78 | 0.0 |
| *Elymus triticoides* W 2741 | 60 | 0.0 | 61 | 0.0 | 49 | 0.0 | 18 | 0.0 | 28 | 3.5 | 55 | 0.0 | 65 | 0.0 |
| *Elymus triticoides* W 3250 | 40 | 0.0 | 59 | 0.0 | 67 | 0.0 | 20 | 5.0 | 49 | 0.0 | 56 | 0.0 | 85 | 0.0 |
| *Hordeum jubatum* var. *caespitosum* F 334 | 106 | 0.9 | 72 | 4.1 | 75 | 0.0 | 66 | 0.0 | 49 | 0.0 | 87 | 1.1 | 52 | 3.8 |
| *Sitanion jubatum* W 3360 | 33 | 18.1 | 62 | 0.0 | 45 | 0.0 | 25 | 0.0 | 17 | 0.0 | 16 | 0.0 | 11 | 0.0 |
| *Cereals:* | | | | | | | | | | | | | | |
| Hard Federation wheat | 40 | 0.0 | 42 | 0.0 | 34 | 0.0 | 32 | 0.0 | 32 | 0.0 | 41 | 17.0 | 81 | 12.3 |
| Kanred × Hard Federation | 47 | 0.0 | 50 | 0.0 | 43 | 0.0 | 43 | 0.0 | 43 | 0.0 | 46 | 21.7 | 84 | 15.4 |
| Prolific rye | 18 | 0.0 | 19 | 0.0 | 24 | 0.0 | 15 | 0.0 | 22 | 9.0 | 25 | 0.0 | 25 | 0.0 |
| Dakold rye | 41 | 0.0 | 37 | 0.0 | 44 | 0.0 | 32 | 0.0 | 29 | 24.1 | 39 | 0.0 | 70 | 0.0 |
| Rosen rye × *Secale montanum* | 24 | 0.0 | 14 | 0.0 | 12 | 0.0 | 12 | 0.0 | 14 | 7.1 | 9 | 0.0 | 6 | 0.0 |

[a] For the sake of brevity species not infected by any of the seven collections of flag smut are omitted from this table.

[b] These collections of flag smut originated as follows:

C–I *Urocystis agropyri* from *Agropyron trachycaulum* Yellowstone Park, Wyoming.
C–H *Urocystis agropyri* from *Hodreum jubatum*, Baker, Oregon.
C–G *Urocystis agropyri* from *Poa ampla*, Yellowstone Park, Wyoming.
C–F *Urocystis agropyri* from *Agropyron repens*, University Farm, St. Paul, Minn.
C–E *Urocystis occulta* from cultivated rye, St. Paul, Minnesota.
C–D *Urocystis tritici* from cultivated wheat, Goldendale, Washington.
C–$D_1$ *Urocystis tritici* from cultivated wheat, Leavenworth, Kansas.

[c] "W" numbers are the accession numbers of the Pullman Nursery Unit of the Soil Conservation Service, U. S. Department of Agriculture.

*Agropyron repens,* respectively have quite different host ranges and probably represent 3 different races. No satisfactory definite explanation can be offered for the behavior of collection C–G, from *Poa ampla.* This collection failed to infect any of the 28 species of grasses (including *Poa ampla* and other *Poa* spp.) inoculated, as well as the cereals. Either all of these were immune from collection C–G, or, what seems more likely, the inoculum did not contain germinable spores.[7]

The 2 collections of *Urocystis tritici,* C–D and C–$D_1$, have previously been shown (8) to represent 2 physiologic races, by their reactions on wheat. This fact is confirmed by their reactions on grasses (Table 3). On the wheat differentials the Washington race is more widely virulent than the Kansas race; whereas, on grasses, the Kansas race is considerably more virulent than the Washington race. A higher percentage of infection was, in fact obtained on *Elymus glaucus* with the Kansas race than on the 2 wheat varieties.

The flag smut of rye did not appear highly virulent on grasses; in fact, it seems that a fair test of the susceptibility of the 28 grass species to all 7 flag smut collections may not have been obtained; as, in general, the percentages of infection are low. Even with the inoculations of *U. tritici* on known susceptible wheats, and of *U. occulta* on susceptible varieties of rye, the percentages of infection were comparatively low, none exceeding 24.1 per cent. This would indicate that some unknown factors may have operated against infection in the entire experiment.

### Cross-inoculation Experiments with Urocystis agropyri, U. tritici, and U. occulta on Cereals and Cereal × Grass Hybrids

In a preliminary test (1940–41) on the susceptibility of wheat and rye to the flag smut of grasses (*Urocystis agropyri*) 32 varieties of wheat and 2 of rye were inoculated with a composite of collections from *Elymus canadensis* and *Agropyron spicatum.* The results were negative. In a second experiment of the same nature (1941–42) 7 varieties of wheat, known to be susceptible to *U. tritici* and 2 varieties of rye susceptible to *U. occulta* were inoculated with *U. occulta* and a composite of collections of *U. agropyri* from various grasses.[8] Good infection resulted on the rye inoculated with *U. occulta;* but, aside from this, the results were negative. Then the experiment was repeated, using a mixture of collections of *U. agropyri* from *Elymus canadensis* and *Agropyron spicatum.* This time, 2 of 30 plants of Kanred × Hard Federation wheat resulting from seed inoculated with *U. agropyri* showed typical flag-smut symptoms. These infected plants were dried and the spores obtained from them were used to reinoculate Kanred × Hard Federation and also *Elymus glaucus,* resulting in 17 and 21 per cent infection, respectively.

[7] The inoculum was not checked for percentage of spore germination. As yet no germinating spores of *Urocystis agropyri* have been observed, although preliminary attempts have been made to induce germination by the technique employed with *U. tritici* and *U. occulta* (11, 13, 14).

[8] Collections C–I, C–H, C–G, and C–F. See footnote b, table 3.

Inasmuch as various cereal × grass hybrids were available through the courtesy of cooperating agencies, a number of these hybrids and their parents were inoculated with *Urocystis tritici* and *U. occulta*. The results are shown in table 4.

TABLE 4.—*The results of inoculating certain cereal × grass hybrids and their parents with Urocystis tritici and U. occulta in the greenhouse*

| Host | *U. tritici* | *U. occulta* |
|---|---|---|
| *Secale montanum* | −−[a] | −− |
| *Elymus condensatus* | − | − |
| Dakold rye × *S. montanum* 3–46. $F_1$ seed | + | + |
| Rosen rye × *S. montanum* 22–12. $F_1$ seed | − | + |
| Prolific rye × *S. montanum* 5–14. $F_1$ seed | + | + |
| Michel's rye | − | + |
| Dakold rye | − | − |
| Mosida wheat | + | − |
| Prolific rye | − | + |
| Rosen rye | − | + |

[a] −−− = no stand obtained.
− = no infection.
+ = typical flag smut symptoms. (Counts were not made and percentages of infection were not calculated.)

As seen in table 4, 2 of the hybrids (Dakold rye × *Secale montanum* $F_1$ and Prolific rye × *S. montanum* $F_1$ were infected with both *Urocystis tritici* and *U. occulta*. It was not surprising to obtain infection on these hybrids with the rye flag smut but the infection with the wheat flag smut was worthy of notice. Repeated attempts to infect the rye parents, Dakold and Prolific rye, with flag smut of wheat have been unsuccessful. This would indicate susceptibility in the other parent, *Secale montanum*. Unfortunately no stand was obtained with this grass, and this possibility was not tested. The susceptibility of *S. montanum* to the flag smut of rye, however, was demonstrated as early as 1907 by Hecke (7), according to Noble (13).

The results of the experiment on which table 4 is based, not the least of which was the infection of Michel's rye with *Urocystis occulta*, prompted a repetition on a slightly larger scale. *Urocystis agropyri* (a composite of collections from *Agropyron, Hordeum,* and *Poa* spp.) was added to the smuts, and other hybrids and parents added to the grasses. The results of this second experiment are presented in table 5.

The results shown in table 5 at least partly substantiate the results shown in table 4. Only one of the rye × *Secale montanum* hybrids was infected with *Urocystis tritici,* and surprisingly enough, it was not infected with *U. occulta*. However, this was not the same hybrid as was used before (Table 4), although the parents are the same. The other hybrid that became infected with both *U. tritici* and *U. occulta* in the previous test was this time not infected by either species. Again Michel's rye showed some infection by *Urocystis occulta*. Three other rye × *Secale montanum* hybrids, not included in the previous test, were infected with the rye flag smut. For the second time, *Elymus condensatus* appeared to be immune from both the

TABLE 5.—*Results of inoculations of cereal × grass hybrids and their parents with Urocystis tritici, U. occulta, and U. agropyri in the greenhouse*

| Host | *Urocystis tritici* | | *Urocystis occulta* | | *Urocystis agropyri* | |
|---|---|---|---|---|---|---|
| | Total plants | Smut | Total plants | Smut | Total plants | Smut |
| | *No.* | *Per cent* | *No.* | *Per cent* | *No.* | *Per cent* |
| *Secale montanum* | --[a] | -- | -- | -- | -- | -- |
| *Elymus condensatus* | 20 | 0.0 | 15 | 0.0 | 30 | 0.0 |
| Dakold rye × *S. montanum* $F_2$ | 47 | 6.8 | 20 | 0.0 | 35 | 0.0 |
| Rosen rye × *S. montanum* $F_2$ | 45 | 0.0 | 15 | 0.0 | 40 | 0.0 |
| Prolific rye × *S. montanum* $F_1$ | 18 | 0.0 | 6 | 0.0 | 18 | 0.0 |
| Michel's rye | 25 | 0.0 | 18 | 5.5 | 35 | 0.0 |
| Dakold rye | 87 | 0.0 | 12 | 0.0 | 20 | 0.0 |
| Mosida wheat | 21 | 0.0 | 26 | 0.0 | 30 | 0.0 |
| Prolific rye | 20 | 0.0 | 12 | 16.6 | 25 | 0.0 |
| Rosen rye | 10 | 0.0 | 8 | 50.0 | 20 | 0.0 |
| Spring rye × *S. montanum* | 40 | 0.0 | 19 | 15.7 | 45 | 0.0 |
| *S. cereale* × *S. montanum*–10 | 12 | 0.0 | 4 | 25.0 | 17 | 0.0 |
| *S. cereale* × *S. montanum*–4 | 1 | 0.0 | 1 | 100.0 | 10 | 0.0 |
| Wheat × *Agropyron elongatum* | 7 | 0.0 | 13 | 0.0 | 20 | 0.0 |
| *Agropyron elongatum* | 30 | 0.0 | 50 | 0.0 | 50 | 0.0 |

[a] -- No stands of *Secale montanum* were obtained.

wheat and the rye flag smuts. Mosida wheat did not this time become infected with *U. tritici*, as it had before.[9]

*Urocystis agropyri* produced no visible symptoms on any of the hybrids and parents tested.

## DISCUSSION

The foregoing results indicate a genetic relationship between the flag smut of grasses, *Urocystis agropyri*, and the flag smut of wheat, *U. tritici*. Possibly the flag smut of rye, *U. occulta*, is also involved, but the latter is morphologically distinct, whereas *U. agropyri* and *U. tritici* are indistinguishable on that basis (4). The established susceptibility of several common grasses to *U. tritici* indicates that native and introduced grasses could very easily support the wheat flag smut in nature, a fact which could eventually become a factor in the control of the disease on wheat. The present results indicate very little susceptibility in wheat to the flag smut of grasses. Still, the slight infection of Kanred × Hard Federation by *U. agropyri* gives a hint that from the nation-wide grass flag smut there could have arisen strains that are capable of attacking wheat in addition to grasses. Thus, the outbreaks of wheat flag smut in the middle west and in the far west may have had an indigenous origin, rather than having been introduced

[9] The parentage of Michel's rye has been reported as *Elymus condensatus* × Mosida wheat, but it has been contended that the parentage is actually rye × *Secale montanum*. If it were a hybrid between wheat and *E. condensatus*, perhaps some susceptibility to wheat flag smut should be manifest, especially since the wheat parent became infected in the first test. Instead, Michel's rye has shown at least some susceptibility to the rye flag smut in both tests and no susceptibility to wheat flag smut. This might be interpreted as additional evidence that Michel's rye is of rye parentage, rather than of wheat. It has behaved, in these experiments, much like the rye × *Secale montanum* hybrids used.

from Australia or elsewhere. The hypothesis finds some support in the high degree of susceptibility of certain common native grasses (*e.g., Elymus canadensis* and *E. glaucus*) to the flag smut of wheat in the United States. Repeated attempts in Australia to infect grasses with the wheat flag smut having been unsuccessful (10), it is possible that a different race is prevalent in that country.

This hypothesis that flag smut of wheat (in the United States) arose as strains of *Urocystis agropyri* capable of attacking wheat may not seem very strongly supported by the results presented in this paper. However, too few collections of the grass flag smut have been studied for the idea to be abandoned without further test.

If the slight infection of Kanred × Hard Federation wheat by *Urocystis agropyri,* described in the results above, is experimental evidence that flag smut of wheat, in the United States at least, represents strains of *U. agropyri* capable of infecting wheat, then the smut resulting from inoculation of Kanred × Hard Federation wheat with the bulk collection of *U. agropyri* might represent a "strain" of *U. agropyri* that had been "screened out" on this wheat variety and that should be capable of continued propagation on the same variety. This is exactly what happened. Where only 6 per cent infection had been obtained when Kanred × Hard Federation was inoculated with the composite collection of *U. agropyri,* 17 per cent infection resulted when this wheat variety was re-inoculated with inoculum made up from the original 6 per cent infection.

It might be argued that there is no greater justification for an hypothesis that flag smut of wheat in the United States derived from the flag smut of grasses than that the converse could have happened. However, the flag smut of grasses has been known in this country for 3 or 4 decades longer than flag smut of wheat and is widely distributed from coast to coast, whereas the wheat smut is localized in a few areas in the middle west and in one small area in the far west.

The present results indicate that within the flag smut of grasses, *Urocystis agropyri,* there probably exist many physiologic races. Three of four collections included in these studies proved to represent different races. These results would seem to strengthen the suggestion recently made (3, 4) that the flag smut of wheat, *U. tritici,* be considered a specialized variety of *U. agropyri.* There does not seem to be any more reason to recognize the flag smut of wheat as a distinct species than any of the other morphologically identical races on Gramineae.

From the results of the cross-inoculation experiments to date it appears that the flag smut of rye has much fewer grass hosts than has that of wheat. Thus far only 2 species of *Agropyron* and 2 of *Elymus* have shown any susceptibility to flag smut of rye; whereas 8 species of *Agropyron,* 3 of *Elymus,* and 1 of *Hordeum* have shown more or less susceptibility to the flag smut of wheat. McAlpine (12) reported *U. occulta* on *Poa caespitosa* Forst. and *Lolium perenne* L. in Australia but these records are subject to suspicion as

referring to *U. agropyri* rather than to *U. occulta*. McAlpine's (12) illustrations of the spore balls of *U. occulta* on *Poa caespitosa* show the spores completely invested by the sterile cells, which would indicate that the smut was *U. agropyri*. McAlpine (12) also stated that *U. occulta* occurs on oats and barley, but did not indicate any authority for such records.

## SUMMARY

The results are reported of cross-inoculation experiments with the flag smut of grasses (*Urocystis agropyri*), the flag smut of wheat (*U. tritici*), and the flag smut of rye (*U. occulta*), and the susceptibility of forage grasses to these smuts.

Twelve species of grasses have been found more or less susceptible to the flag smut of wheat. These are *Agropyron caninum, A. dasystachyum, A. desertorum, A. inerme, A. repens, A. semicostatum, A. spicatum, A. trachycaulum; Elymus canadensis, E. glaucus, E. triticoides;* and *Horedum jubatum* var. *caespitosum*.

Four species of grasses have exhibited more or less susceptibility to flag smut of rye. These species are *Agropyron caninum, A. inerme; Elymus canadensis,* and *E. triticoides*.

There is evidence of considerable host specialization in the flag smut of grasses and many physiologic races probably occur in the United States. Three of 4 collections studied proved to represent different races.

One variety of wheat (Kanred × Hard Federation C.I. 10092) exhibited slight susceptibility to *Urocystis agropyri* in 1 out of 3 trials, and the smut thus produced was easily propagated on the same wheat variety. Wheat and rye seem to be immune from *U. occulta* and *U. tritici*, respectively. Rye seems immune from *U. agropyri*.

It is considered that the present results indicate a genetic relationship between *Urocystis agropyri* and *U. tritici*. *U. occulta* may also be involved. Considering the number of grasses that already have exhibited at least slight susceptibility to flag smut of wheat, it is suggested that flag smut of wheat may have arisen as strains of *U. agropyri* capable of attacking wheat, and that, therefore, the outbreaks of wheat flag smut in the United States may have an indigenous origin from the widespread flag smut of grasses, rather than having been introduced from abroad.

The data presented herein concerning the host relationships of the three flag smuts substantiate the morphological identity already demonstrated and strengthen the recommendation that the flag smut of grasses and the flag smut of wheat be consolidated under the binomial having priority, which is *Urocystis agropyri* (4).

WASHINGTON STATE COLLEGE,
PULLMAN, WASH.

## LITERATURE CITED

1. BRITTLEBANK, C. C. Seed borne diseases, "take-all and flag-smut." Jour. Dept. Agr. Victoria 19: 447. 1921.
2. CLINTON, G. P. Ustilaginales. North Amer. Flora 7 (1): 1–82. 1906.

3. FISCHER, GEORGE W. Infection of forage grasses with flag smuts of wheat, rye, and grasses (*Urocystis tritici, U. occulta,* and *U. agropyri,* respectively). (Abstract) Phytopath. 32: 4–5. 1942.
4. ————. Some evident synonymous relationships in certain graminicolous smut fungi. Mycologia 35: (in press). 1943.
5. GRIFFITHS, MARION A. Experiments with flag smut of wheat and the causal fungus, *Urocystis tritici* Ecke. Jour. Agr. Res. [U. S.] 27: 425–450. 1924.
6. HEALD, F. D., and C. S. HOLTON. Flag smut of wheat found in Washington. U. S. Dept. Agr. Plant Dis. Rptr. 24: 382. 1940. (Mimeographed.)
7. HECKE, L. Die Triebeninfektion bei Brandpilzen. Zeitschr. Landw. Versuchs. Oesterr. 10: 572–574. 1907.
8. HOLTON, C. S., and A. G. JOHNSON. Physiologic races in *Urocystis tritici.* Phytopath. 33: 169–171. 1943.
9. HUMPHREY, H. B., and A. G. JOHNSON. Take-all and flag smut, two wheat diseases new to the United States. U. S. Dept. Agr. Farmer's Bull. 1063. 1919.
10. JARRETT, PHYLLIS H. Investigations on flag smut of wheat. Jour. of the Council for Sci. and Indus. Res. 5: 165–169. 1932.
11. LING, LEE. Factors affecting spore germination and growth of *Urocystis occulta* in culture. Phytopath. 30: 579–591. 1940.
12. MCALPINE, D. The smuts of Australia. 288 pp. (Melbourne.) 1910.
13. NOBLE, ROBERT J. Studies on the parasitism of *Urocystis tritici* Koern. the organism causing flag smut of wheat. Jour. Agr. Res. 27: 451–489. 1924.
14. STAKMAN, E. C., R. C. CASSELL, and M. B. MOORE. The cytology of *Urocystis occulta.* Phytopath. 24: 874–889. 1934.
15. TISDALE, W. H., G. H. DUNGAN, and C. E. LEIGHTY. Flag smut of wheat, with special reference to varietal resistance. Ill. Agr. Exp. Stat. Bull. 242. 1923.
16. ————, C. E. LEIGHTY, and BENJAMIN KOEHLER. Further studies on flag smut of wheat. U. S. Dept. Agr. Circ. 424. 1927.
17. ZUNDEL, G. L. Additions and corrections to the Ustilaginales. North Amer. Flora 7: (14) 971–1045. 1939.

# ESTIMATION OF THE LEAF AREA OF POTATO PLANTS FOR PATHOLOGICAL STUDIES

J. G. Bald[1]

(Accepted for publication November 16, 1942)

## INTRODUCTION

Measurement of the leaf area of experimental plants is normally a laborious process, often involving the destruction of the plants on which measurements are taken. A method recently has been devised[2] that greatly facilitates such measurements, that can be applied without injury to experimental plants, and that is as accurate as some of the more laborious methods. A series of standards is obtained by making tracings of leaves graded in size from the smallest to the largest, the tracings are numbered serially, and each leaf on the plants to be measured is matched with the standard nearest in size. The area of the leaf is recorded by applying to it the number of the corresponding standard.

The principle of this method has been modified and extended to the measurement of whole plants. In an experimental plot, the areas of all leaves on a few plants differing in size are compared with a set of standard leaves, the total area for each plant is referred to a scale covering the whole range of plant size, and the plants are given numbers according to their position on the scale. They are then used as standards for estimating the size of all plants in the plot. In practice, once the reference plants are given numbers according to the scale, the erection of a full series of standards becomes more an act of judgment and memory than of comparison.

This method, if properly applied, is very quick and surprisingly accurate. A practised operater, after establishing the scale of sizes by measurement and comparison, can walk through a plot calling the numbers that indicate plant size almost as quickly as they can be written down. By far the most laborious part of the method is the assembly, conversion, and analysis of the data.

The adaptability of the method has been proved by applying it to potato plots containing a number of varieties varying widely in growth habit. After a little practice, the mental adjustment necessary when passing from one variety to another becomes almost entirely mechanical.

The method is subject to two kinds of errors, errors of judgment, and errors arising from the grouping of the leaf areas into classes. The extent of the former may be tested in two ways: by measuring the areas of plants with a set of leaf standards after the plants have been rated, and by making and comparing duplicate ratings. The errors of grouping are equivalent to those involved in the grouping of data for statistical analysis, and will be

[1] Pathologist, Division of Plant Industry, C.S.I.R.

[2] Thirumalachary, N. C. A rapid method of measurement of leaf areas of plants. Ind. Jour. Agr. Sci. 10: 835–841. 1940.

small, provided sufficient classes are established, and the data are well distributed amongst the classes.

The success or failure of the method depends on how great are the errors of judgment in comparison with the accuracy demanded by the nature of the experiment.

## DESCRIPTION OF THE EXPERIMENTAL PLANTS

Leaf areas for whole plots of potato plants were needed for two types of experiment, i.e., on transmission of leaf roll by aphids and on yield. The method of obtaining the leaf area was developed by trials mainly on a block of plants set out for investigations on the aphis transmission of leaf roll. The majority of examples and results given in the following description are drawn from the data for this block.

The block consisted of 864 plants in 16 rows of 54, and was surrounded by check rows to eliminate edge effects. The experiment was designed to include four varieties, Early Carman,[3] Western Australian Delaware (early varieties), Up-to-Date, and Tasmanian Brownell. The statistical unit subplot was intended to consist of 5 plants of the same variety and an Up-to-Date plant known to be infected with leaf roll, but when the plants were growing, it was found that the healthy Up-to-Date stock contained about 40 per cent. of another variety, probably Great Scot.

The first attempt to obtain a figure for leaf area was semi-quantitative, and was made on October 9th. Five other ratings were made, the last on December 11th. At this time, both early and late varieties had completed the period of most rapid vegetative growth and were developing tubers.

TABLE 1.—*Ratings and corresponding leaf areas for the scale used in estimating areas of potato leaves*

| Rating | Log leaf area | Leaf area |
|---|---|---|
| | *sq. cm.* | *sq. cm.* |
| < 1 | 1.0 | 10 |
| 1 | 1.32 | 21 |
| 2 | 1.425 | 27 |
| 3 | 1.53 | 34 |
| 4 | 1.635 | 43 |
| 5 | 1.74 | 55 |
| 6 | 1.845 | 70 |
| 7 | 1.95 | 89 |
| 8 | 2.055 | 114 |
| 9 | 2.16 | 145 |
| 10 | 2.265 | 184 |
| 11 | 2.37 | 237 |
| 12 | 2.475 | 299 |

## DEVELOPMENT OF A STANDARD FOR LEAVES

In obtaining a standard series of outlines, a number of leaves varying in size, shape, and variety, were picked from plants of the same varieties as

[3] For a discussion of the origin of these varieties, see C.S.I.R. (Australia) Pamphlet 106.

were in the experimental block, and contact prints were made of them on photographic paper. Reasonably clear prints were obtained, without injuring or distorting the leaves, by placing them with the midrib lying in a channel between two sheets of glass placed slightly apart on the screen of a printer. Pressure could then be exerted to bring the paper and the leaf into contact without crushing the leaf.

When the prints of a number of leaves were made, they were laid out on a bench. Three of the smallest were chosen because they appeared to belong to an evenly ascending series of sizes and rated 1, 2 and 3. Amongst the prints available there did not appear to be a fourth that was bigger than 3 by the same amount as 3 was bigger than 2 or 2 than 1. Similarly, there was none that seemed of a size to be rated 5, but there was one that could be rated 6; and from there the series was continued up to 8 by the choice of two successively larger leaves. The areas of the chosen prints were measured with a planimeter and compared with the ratings. The results are shown in figure 1, a. Other attempts at rating gave similar results.

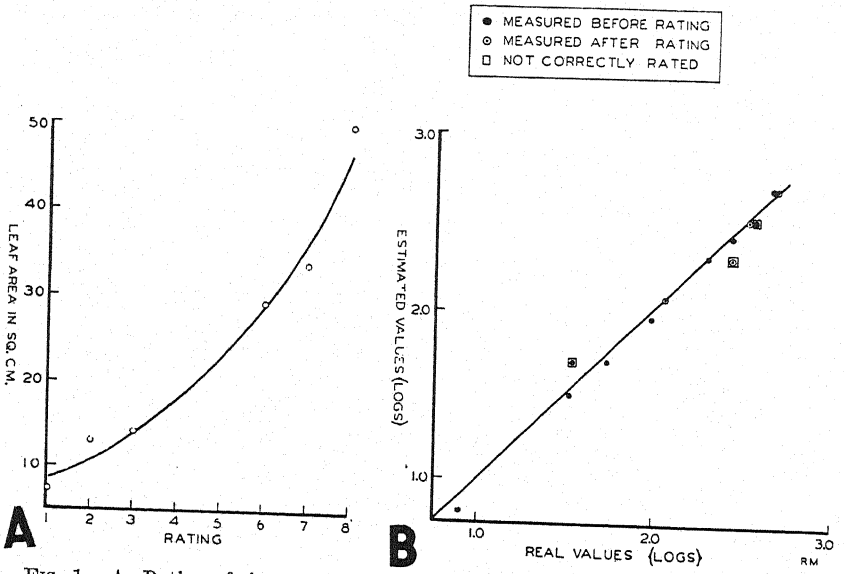



FIG. 1. A. Rating of six potato leaves and their areas as measured with a planimeter. The curve passing through the points was obtained from a straight line fitted to the values for log leaf area by the method of least squares. B. Comparison of leaf areas of whole plants which were both measured by means of the leaf standards (real values) and rated according to the standard scale given in table 3 (estimated values).

It was obvious that the points did not fit well into an arithmetic series, but when logs of the areas for a series of ratings were plotted, the values fell about a straight line. Apparently the mind judges leaf area on a basis of proportionality, *i.e.*, by the addition or substraction of equal relative increments rather than of absolute increments.

This basis appeared to offer a convenient means of compressing a wide range of sizes into a workable scale. Prints of leaves were measured until

samples were obtained that fitted into the 12-point scale shown below (Table 1). This scale was not too coarse for the kind of work contemplated, and it was quick in use and convenient to handle. The prints chosen were pasted on cloth sheets, supported on a stiff board, and bound at the top.

No leaves were found that were too large to be rated 12, but there was a number smaller than the leaf rated 1. An area of 10 sq. cm. was ascribed to all these leaves, and to the whorls of young leaves at the growing tip.

To remove the chance of bias in matching the leaves because of differences in shape, the more dissected types were chosen for odd-number ratings, and the more rounded and less dissected types for the even numbers. A leaf of any shape could then be placed for comparison between two somewhat similar outlines. The alternation of different shapes on the scale also helped to obviate the need for constructing different scales for different varieties.

### DEVELOPMENT OF A STANDARD FOR PLANTS

The first two ratings of this block of plants were made on October 9 and 16. Plants were noted as "not yet up," "aboveground but the first young leaves not fully expanded," and "the first young leaves expanded." This classification was given quantitative expression by assuming that the second and third classes were equivalent to the first two on a scale developed for the third rating made on October 24 (Table 2). The areas so obtained were not grossly incorrect, and they gave a reasonable picture of the relative leaf areas of the different varieties at each date.

On October 24 the plants were rated on a 10-point scale by choosing the largest and some of the smallest plants, rating them 10 and 1, and ascribing what seemed appropriate values to plants of intermediate size. After the rating was made, plants of various sizes and varieties were chosen at random, and the leaf areas were found by measurement with the leaf standards. The results were somewhat irregular, but they did not suggest that estimation of the leaf area of whole plants was made on a simple basis of proportionality.

A week later, the largest plant had nearly doubled its leaf area so an attempt was made to double the intervals on the scale for rating. The result was fairly successful; the largest plant was rated 9 and the intermediate ratings were about double the previous ratings. Again the estimates of leaf area could not be fitted on a simple basis of proportionality, but neither did the increments appear always to be equal on an arithmetical scale although they were near enough to equality to make the use of an arithmetical scale permissible. The values shown in table 2 were used in converting the ratings to areas. The values for 24.10.42 and 31.10.42 were obtained from straight lines fitted to experimental values on a graph of the measured leaf area plotted against the rating previously given to the measured plants. Immediately after the ratings in the plot were completed, a number of plants of various sizes were chosen at random, and their leaf areas measured by means of the leaf standards. For each plant the area so obtained was

plotted against the rating. Straight lines were fitted to the points on the graph.

The lines that gave the values in table 2 varied very slightly, but far from significantly, from those obtained by the method of least squares: the increments gave the nearest rounded figures to the figures calculated.

TABLE 2.—*Scales used in converting ratings of plant size into leaf areas. Leaf Roll Transmission Exp. Block 1, 1941–42*

| Date: | 24.10.41 | | 31.10.41 | | | 12.11.41 | |
|---|---|---|---|---|---|---|---|
| Rating | [a]Area in sq. dm. | | Rating | [a]Area in sq. dm. | | Rating | Area in sq. dm. |
| | 3 varieties | Brownell | | 3 varieties | Brownell | | |
| 1 | .5 | 0.4 | 1 | 2.25 | 1.25 | 1 | 6.2 |
| 2 | 3.0 | 2.0 | 2 | 7.25 | 5.0 | 2 | 16.5 |
| 3 | 5.5 | 3.6 | 3 | 12.25 | 8.75 | 3 | 18.7 |
| 4 | 8.0 | 5.2 | 4 | 17.25 | 12.5 | 4 | 21.4 |
| 5 | 10.5 | 6.8 | 5 | 22.25 | 16.25 | 5 | 24.6 |
| 6 | 13.0 | 8.4 | 6 | 27.25 | 20.0 | 6 | 28.3 |
| 7 | 15.5 | ..... | 7 | 32.25 | ..... | 7 | 32.5 |
| 8 | 18.0 | ..... | 8 | 37.25 | ..... | 8 | 37.0 |
| 9 | 20.5 | ..... | 9 | 42.25 | ..... | 9 | 43.0 |
| 10 | 23.0 | ..... | | | | 10 | 49.0 |
| | | | | | | 11 | 56.0 |
| | | | | | | 12 | 64.0 |
| | | | | | | 13 | 74.0 |
| | | | | | | 14 | 85.0 |

[a] There were no significant differences in the leaf areas of plants of Early Carman, Delaware, and Up-to-Date that were equally rated.

The estimation of leaf area for one variety, Brownell, showed a bias resulting in ratings for the larger plants that were too high, *i.e.*, the plants were judged to have a greater leaf area than they really had. A separate scale was used for this variety in converting the ratings to areas. In later estimates of leaf area the bias was corrected, and it was possible to use the same scale for all plants.

The rating of November 12 provided a possible explanation for some of the apparent irregularities in previous estimates. It was decided to use a 15-point scale; in fact, the highest rating given was 14. Leaf areas of a number of plants, measured after the estimates of leaf area had been made, were plotted against the ratings for the same plants. From 2 to 14 they formed an evenly ascending series fitting the conception that leaf areas for whole plants were judged on a basis of proportionality, but the mean area of plants rated 1 fell far below what would have been expected from the figures for the larger plants. The scale for November 12 was obtained by fitting a straight line to the points obtained by plotting log leaf area of a number of rated plants against the ratings given them. In class 1 were grouped all plants with a leaf area less than about 12 sq. dm.

Reexamination of the data for the estimates of October 24 and 30 showed that there was an alternative to the arithmetic scale for converting ratings to areas, an alternative that eliminated some of the variability shown in the

measurements of the leaf areas of rated plants. The data were fitted quite well by assuming that the area of plants both above and below 12 sq. dm. was estimated on a basis of proportionality, but that the logarithmic increments between classes for the smaller plants were greater than for the larger plants. Classes 6 to 10 at the third rating (October 24), 3 to 9 at the fourth rating (October 31), and 2 to 14 at the fifth rating (November 12) were in the range where logarithmic increments between classes were relatively small. It is possible that the mind, faced by the problem of rating plants over a relatively enormous range of sizes, reduced the scale for estimating leaf area when the increments in actual leaf area became too large, but continued to make estimates on a basis of proportionality.

TABLE 3.—*Scale for estimating size of potato plants*

| Rating | Log mean | Mean | Limits |
|---|---|---|---|
| | | *sq. dm.* | *sq. dm.* |
| 1 | 0.86 | 0.7 | 0.55– 0.95 |
| 2 | 0.10 | 1.25 | 0.95– 1.65 |
| 3 | 0.34 | 2.2 | 1.65– 2.9 |
| 4 | 0.58 | 3.8 | 2.9 – 5.0 |
| 5 | 0.76 | 5.8 | 5.0 – 6.6 |
| 6 | 0.88 | 7.6 | 6.6 – 8.7 |
| 7 | 1.00 | 10.0 | 8.7 – 11.5 |
| 8 | 1.12 | 13.2 | 11.5 – 15.1 |
| 9 | 1.24 | 17.4 | 15.1 – 20.0 |
| 10 | 1.36 | 22.9 | 20.0 – 26.3 |
| 11 | 1.48 | 30.2 | 26.3 – 34.7 |
| 12 | 1.57 | 37.2 | 34.7 – 39.8 |
| 13 | 1.63 | 42.7 | 39.8 – 45.7 |
| 14 | 1.69 | 49.0 | 45.7 – 52.5 |
| 15 | 1.75 | 56.2 | 52.5 – 60.3 |
| 16 | 1.81 | 64.6 | 60.3 – 69.2 |
| 17 | 1.87 | 74.1 | 69.2 – 79.4 |
| 18 | 1.93 | 85.1 | 79.4 – 91.2 |
| 19 | 1.99 | 97.7 | 91.2 –105.0 |
| 20 | 2.05 | 112.0 | 105.0 –120.0 |
| 21 | 2.11 | 129.0 | 120.0 –138.0 |

## STANDARD METHOD OF RATING PLANTS

It was decided to fix a scale to which all plants rated could be referred, and to design it according to this hypothesis. By using one scale much of the labor and many of the errors likely to result from the use of diverse scales would be avoided. On the other hand, in order to use such a scale plants of known size had to be found and used as standards for every series of ratings made. The scale finally chosen is shown in table 3. It covers a range from 0.7 to 129 sq. dm.; the former represents the leaf area of a young plant almost as soon as the first leaves have expanded above ground and the latter a large mature plant. For plants of a leaf area within the range between $\frac{1}{2}$ sq. dm. to 5 sq. dm. the unit difference between ratings on the logarithmic scale is 0.24, which implies that a unit increase in the rating corresponds to an addition of $\frac{3}{4}$ of the previous leaf area. If this scale were

maintained for bigger plants the increments of leaf area would become too large; so, between the ratings 5 and 11, the logarithmic rate is reduced to 0.12. This means that a unit increase in rating is made for an increase of about one-third in size. For plants of an area greater than 35 sq. dm. the rate of increase for each rating is reduced to about 15 per cent. With very large plants, even this is a large increment.

The usual procedure in rating a block of plants was as follows. The leaf standards, *i.e.*, the prints of the leaves of known standard size, and the scale of sizes for plants were taken into the block of plants to be measured. There was generally an assistant to write down the ratings as the observer gave them. The observer chose one of the biggest plants: from previous experience he might decide it had a leaf area of about 12, according to the rating of the fixed scale. Beginning with the bottom leaf of one shoot, each leaf on the plant was systematically compared with the leaf standards and rated. Immediately the plant was finished, the leaf ratings were converted to areas and the leaf area of the whole plant was found. The rating appropriate to the leaf area was then found from the scale for plants. Instead of 12, the correct rating might be 14. Then one of the smaller plants would be chosen; its rating might be guessed as 4. Perhaps, after measurement, its true leaf area would be found to lie near the border line between ratings 5 and 6 within the range of areas rated 5. Intermediate plants would be measured until the preliminary guesses were found substantially correct. When the leaf areas extended from, say, ratings 2 to 14, perhaps 7 plants would be measured, and intermediate sizes interpolated mentally between those fixed by measurement. Having made a careful examination of the standards, the observer began at one corner of the plot, walking along each row in succession, calling a number for each plant. The assistant wrote this number in the appropriate place on a cyclostyled plan of the plot.

When the rating was complete, a few plants were chosen at random in the plot, their leaf areas measured with the leaf standards, and compared with the ratings already made. The plants originally used as standards also served as checks on the ratings, provided they were well distributed throughout the plot. In a plot of any size, these plants were practically never recognized when the systematic rating of plants was being made. Quite apart from the difficulty of recognizing one particular plant in hundreds the act of rating demanded a degree of concentration that prevented other observations being made at the same time.

### ERRORS IN ESTIMATION

The first attempts to use the fixed scale were not altogether successful; but, with practice, the accuracy of the estimations improved beyond that of ratings made before it was devised.

An example of the accuracy attained is illustrated in figure 1, B. These data were obtained from a block of plants set out to see if any differences existed between 25 tuber lines from a commercial stock of the variety Factor

(Up-to-Date). The block contained 600 plants. The leaf areas of 9 plants in various parts of the block were measured to provide standards of reference, the rating of all plants was made, and the leaf areas of 4 more were

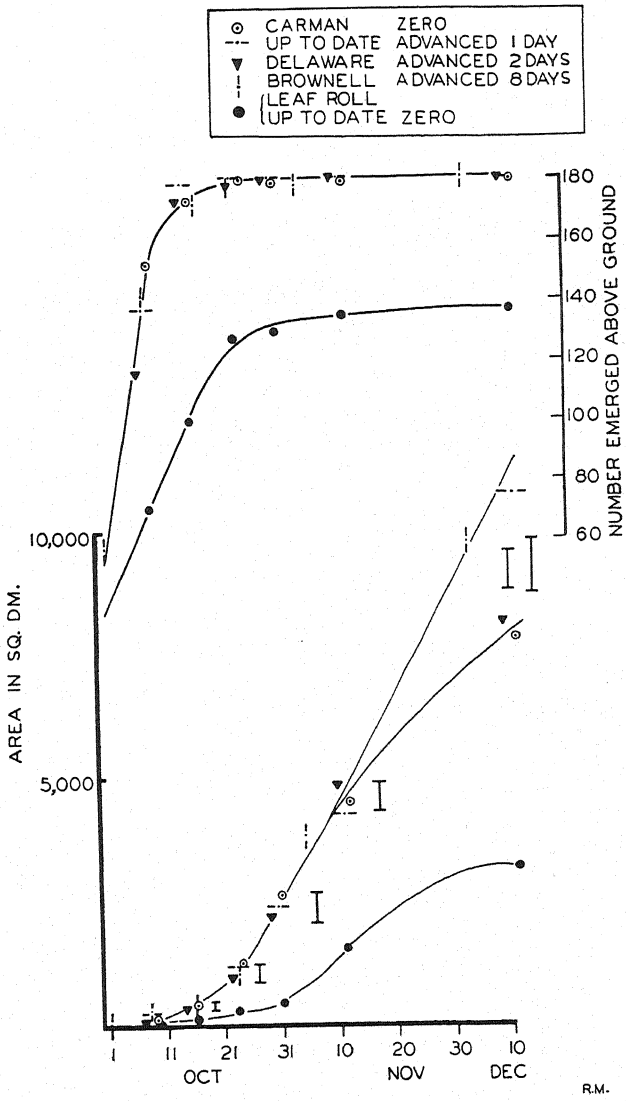



FIG. 2. Emergence and increase of total leaf area for healthy plants of four varieties of potato and one infected with leaf roll. The figures for emergence of the healthy plants were adjusted on the time scale until all varieties fell on the same frequency curve, and equivalent adjustments were made when plotting the leaf areas. Significant differences between leaf areas are shown as vertical lines; for the last rating significant differences between single varieties and paired varieties are both shown.

measured afterwards. The 9 plants first measured were not recognized when they were rated.

The estimates of leaf area for plants measured before and after the rat-

ings were equally reliable. Ten of the 13 plants were correctly rated, the rating of 2 plants was high, and of the other one low. The total leaf area of the 13 plants was, according to the measurements, 129.8 sq. dm., and according to the estimates, 123.1, a difference of 5 per cent. For comparison with these two figures, a third total, 127.2, was obtained by attributing to each plant the mean area for the class into which it fell according to the measurements. This figure is 2 per cent below the total obtained by summing the actual measurements of leaf area. Of the difference of 5 per cent, this 2 per cent may be attributed to errors due to grouping the data, and 3 per cent to errors due to incorrect rating. No bias was introduced into the results by the method of rating, and the breaks in the scale did not affect the accuracy of estimation.

The consistency of rating may be illustrated by an earlier estimate of leaf area from the same experiment. This estimate was obtained before all the plants in the block had emerged from the soil and before the fixed scale was devised. A 6-point scale was used, and the plants barely aboveground or not yet up were rated 0. Two independent ratings were made, one in the morning and one in the afternoon of the same day. About 70 per cent of the plants were given the same rating on each occasion, and all the remainder, except about one per cent, differed by 1 point on the 6-point scale. The combined ratings for unit sub-plots of 4 plants, of which there were 150 in the block, were used in calculating the correlation between the two estimates of leaf area. A value of $r = 0.968$ was obtained, which shows the high degree of association between the two estimates. The mean ratings for the 150 sub-plots were 7.35 and 7.24, *i.e.*, they differed by only 1.5 per cent.

From these results it appears that the method of estimating the leaf area of plants in field plots may be made accurate and reliable, and that the errors of estimation can be reduced below those due to the usual causes of variability affecting the results of field experiments.

## APPLICATION OF THE METHOD TO A FIELD EXPERIMENT

A short description of the block of plants mainly used in the development of the method of estimating leaf areas was given earlier in this paper. The leaf area was estimated in order to give a basis for calculating the development of the total aphis population in the block. The results are also of interest as a study in the vegetative growth of several varieties of potatoes and of diseased and healthy plants, although they cover only part of the whole growing period.

Healthy plants consisted of 5 varieties, one of which (probably Great Scot) occurred as rogues amongst another variety (Up-to-Date). As there were no significant differences between them in leaf area over the period when ratings were made, Up-to-Date and Great Scot together are counted as one variety. The diseased plants were of the variety Up-to-Date infected with leaf roll. There were 6 plots in the block of experimental plants, and each plot contained 24 sub-plots, 6 for each variety. There was a leaf roll plant in every sub-plot.

Leaf areas of all plants at 6 different dates were obtained from the ratings, by using appropriate scales, already described. Plot totals for each variety and for the leaf-roll plants were calculated and used as the basis for statistical analysis. The block totals for the different varieties were plotted against time, and the curves were examined to discover what differences existed between them. At first they seemed very different, but part of the difference was obviously due to the late emergence of the variety Brownell of which the seed tubers were not well sprouted at planting. A better comparison was obtained by shifting the curves along the time scale until the lower portions coincided.

To obtain an estimate of the displacement on the time scale for each variety, the number of plants aboveground on the dates when estimates of the leaf area were made were plotted, and curves drawn through them. The most advanced variety was Early Carman: on October 9, 151 of 180 plants had emerged aboveground. Judging by the date on which the curves for the other varieties passed through the point representing the emergence of approximately 150 plants, the variety Up-to-Date (including Great Scot) was one day behind Early Carman, Delaware was 2 days, and Brownell was 8 days. The curve for the leaf roll plants showed that germination was more irregular and spread over a much longer period than for the healthy plants; therefore, no attempt was made to use it as a measure of emergence.

The data for emergence and leaf areas were plotted again (Fig. 2), the 3 varieties other than Early Carman being advanced on the time scale, Up-to-Date by 1 day, Delaware by 2 days, and Brownell by 8 days. There is a close coincidence of the curves for germination. The growth curves also agree until the stage represented by the date November 12 for Early Carman. Later, there is a divergence in the growth of the early varieties, Early Carman and Delaware, from the later varieties, Up-to-Date and Brownell. The rate of expansion of the leaf area of the early varieties decreased very rapidly from the onset of flowering. Flowering began about November 12, when also, presumably, the tubers began to form. The corresponding period for Up-to-Date and Brownell began not long before the last rating was made on December 11.

The only irregularities in the lower section of the curves occur among the readings for November 12, where a smooth curve can barely be fitted to the points within the range of significant differences. This variation probably has little significance, and may merely represent imperfect adjustment on the time scale. The differences on December 12 between early and later varieties are highly significant, whatever small adjustments are made.

The uniformity of growth of the different varieties in the early stages of development is the more surprising, because each curve represents a population of potato plants emerging over a period, and varying considerably in size and vigor. Also, during November and December a number of originally healthy plants exhibited the first signs of infection with leaf roll. To

attain such uniformity it is suggested the following conditions must have been satisfied:

1. The aggregate size of the rudimentary organs of root and shoot must have been of the same order for the populations of healthy plants of all varieties. When the tuber pieces were prepared for planting this plot, they were cut as nearly as possible to a uniform size, and this may have helped produce uniform development of roots and shoots.

2. After the plant emerged, all 4 varieties grew in a similar manner, in spite of morphological differences. Until flowering they behaved as one variety; afterwards, they behaved as two.

3. The differences of leaf area of early and late varieties are due to the incidence of whatever stimulus caused the diversion of metabolic energy from vegetative growth to the formation of flowers and tubers. It did not depend on differences in metabolic efficiency.

4. In the first stages of infection with leaf roll, infection did not greatly affect the growth of the plant.

It is plain from the curves in figure 3 that the emergence and growth of Up-to-Dates were greatly influenced by long established infection with leaf roll. Emergence continued over a longer period, and far less than 100 per cent of the seed tubers produced plants. Growth was slower and less regular than for healthy plants of the same and other varieties. A few comparatively vigorous plants were produced, but, in most, symptoms of rolling and lack of vigor appeared very early. The plants remained stunted for some time. Many appeared to become more vigorous during early November, and this is reflected in the rise of leaf area between October 31 and November 12. The reason for the very slow growth at first appeared to be that internal necrosis and thin sprouts were common amongst the seed tubers. They represented conditions very unfavorable for the transport of food materials to the young plant, and their use in the development of new tissues.

## SUMMARY

A method has been evolved whereby the leaf areas of groups of potato plants have been estimated with an accuracy ordinarily assumed to be attainable only by direct measurement.

The estimation is quickly enough made to allow the measurement of comparatively large blocks of potato plants within a few hours.

An example is given of the application of the method to a block of 864 plants laid down as part of an experiment on the transmission of leaf roll. The rates of increase in leaf area of early and late varieties included in the block were similar until the early varieties began to flower; thereafter, they fell below those of the late varieties.

Long-standing infection with leaf roll greatly reduced the rate of increase in leaf area.

DIVISION OF PLANT INDUSTRY,
CANBERRA, A.C.T., AUSTRALIA.

# BOREON DEFICIENCY IN THE OLIVE

C. Emlen Scott, H. Earl Thomas, and
Harold E. Thomas

(Accepted for publication March 12, 1943)

Because of the relatively high boron content of many California soils and waters (4, 5, 7, 9), it has seemed improbable that any considerable agricultural areas of the State would be found deficient in that element. Thus far only a few small areas have been demonstrated to be affected by such a deficiency. However, a number of soils of the State have been found "inherently low" in boron (12). At any rate, with the number and diversity of disorders to be diagnosed among tree fruits it has become more or less habitual to test all of the well-known minor elements in cases where no good clue is provided by the symptoms. In this way, a considerable area was found in Butte County, California, in which olive trees (*Olea europea*) responded strikingly to borax or boric acid.

## HISTORY

In 1917, Horne (6) described an "unusual disease" of the olive and stated ". . . the disease was called exanthema on account of a certain fancied resemblance to the exanthema or Florida dieback of citrus trees." Horne recognized an association with defects in drainage but noted that excess water did not always produce exanthema symptoms. His description includes withering of tips of shoots resulting in bushy growth, dieback of branches for several feet and puffed bark. The notion persisted for a number of years that these trees were suffering from exanthema chiefly because of resemblance of symptoms to those of deciduous and citrus trees. Smith and Thomas (11), in 1928, stated that olive growers reported beneficial results from copper sulphate. However, Henry P. Everett, County Agent of Butte County, made copper sulphate injections into trunks of affected olive trees in 1938 and 1939, but failed to see any benefit.

In February, 1941, the senior writer, with Mr. Everett, injected olive trees with copper sulphate and also with boron, manganese, and zinc on a small scale in two orchards. The 4 branches receiving boron showed improvement by July, and the contrast became greater as the deficiency symptoms became more pronounced during late summer and fall. The results of these tests and other injections, sprays, and soil treatments made in 1941 and 1942, most of them with the cooperation of Mr. Everett, are reported herein.

## DISTRIBUTION OF BORON DEFICIENCY

All of the tests of boron on olive thus far completed were made in the Wyandotte district, Butte County. The olives in this area are grown on Aiken clay loam in rolling foothills. Of the 4,000 acres of olives in Butte County over 3,000 are of the Mission variety. Most of the tests have been

made on this variety but the few trees of the Manzanillo and Barouni varieties, under observation in the district, exhibit essentially the same symptoms. Symptoms are also present in olive trees in the Thermalito district near Oroville, Butte County, and near Loma Rica in adjoining Yuba County.

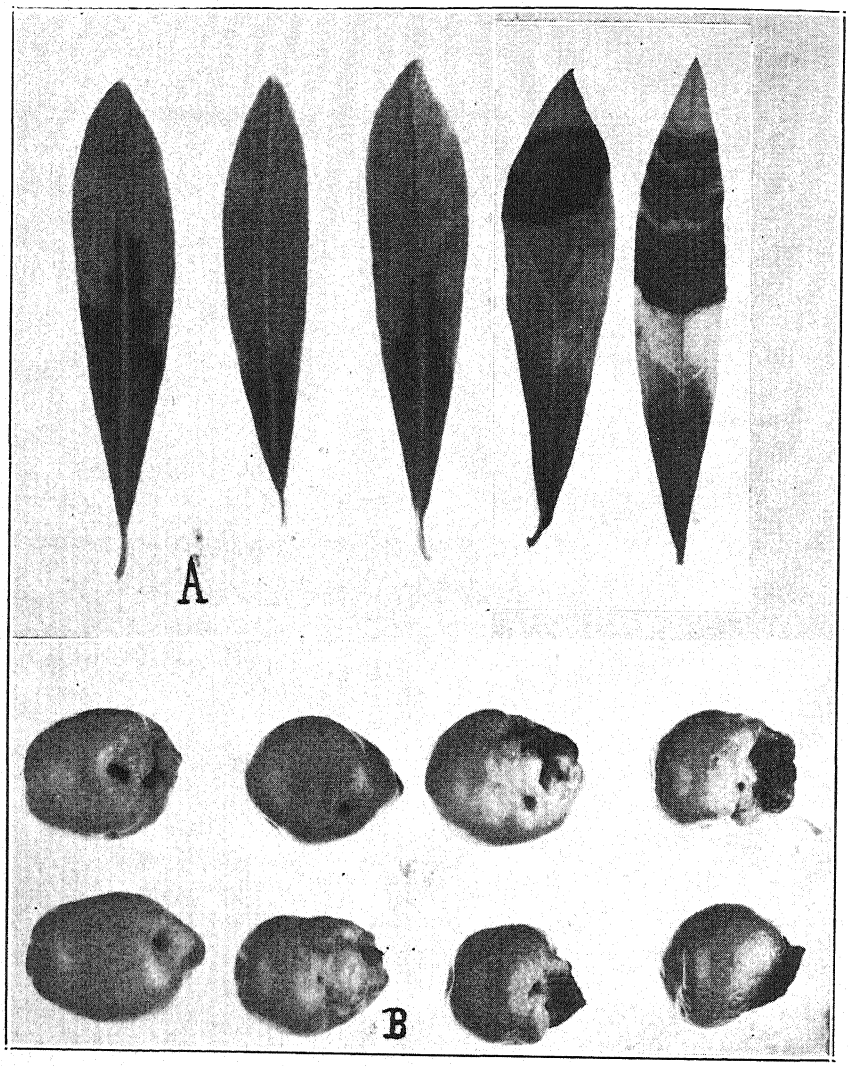



FIG. 1. A. Chlorosis of apical portions of olive leaves and tip necrosis in two leaves at right. B. Pitting and malformation of fruit.

Leaf symptoms indistinguishable from those of trees that respond to boron also have been seen on olive trees in Alameda, Marin, Napa, Sonoma, Santa Clara, and Santa Cruz Counties. In these coastal counties fruit malformation was less common. On the other hand, fruit symptoms but no leaf

symptoms are present in 2 Tulare County orchards in the interior San Joaquin Valley. Boron injection tests have been made in these areas but the results are not yet available. All told, these symptoms have been seen on olive trees growing in a half dozen or more soil types.

## SYMPTOMS

### Leaf Symptoms

In the Wyandotte district all trees known to be deficient in boron bear some leaves with chlorotic tips (Fig. 1, A). This symptom is quite distinctive, and, since the olive is evergreen and retains its leaves for several seasons, it is present at all times. During fall and winter some leaves of the current season's growth become pale green and some of them eventually bright yellow at the apical end. This chlorosis commonly involves $\frac{1}{3}$ to $\frac{1}{2}$ of the blade, the base remaining normal in color. In rare cases a mere trace of green remains at the base of the blade. This leaf symptom is readily distinguished from the aspect of senile leaves, most of which become yellow all over prior to abscission. Some leaves, which have been chlorotic for some months, develop necrotic tips that bear a zonate pattern (Fig. 1, A). Foliage tends to be sparse on severely affected trees (Fig. 2, B).

### Unfruitfulness

Olive trees with mild to moderate leaf and twig dieback symptoms appear to blossom and set fruit normally. Then most of the immature fruits may drop in July and August. The great crop increase on such trees when supplied with adequate boron seems to result mainly from preventing fruit drop rather than from any immediate effect on blossom production. Severely affected trees blossom sparsely.

### Defective Fruit

Trees known to be low in boron generally produce some malformed or defective fruit (Fig. 1, B). The pitting, shrivelling, and drying that usually involve only the apical half or less, is locally known as monkey face. All of the flesh under the deep pits and shrunken apical ends is necrotic. Pitting and shrivelling may occur alone or together on the same fruit. On severely malformed fruits pitting also may occur near the stem end. Defective fruit has been seen on the Mission, Manzanillo, and Barouni varieties. Typical counts of fruit of affected trees show a range from 0 to 40 per cent, with individual branches up to 70 per cent.

### Dieback and Excess Branching

Severely affected trees present a distinct appearance of short bunchy growth (Fig. 2, A) with only an occasional shoot a foot or more in length. Up to early summer, shoots supplied with adequate boron cannot be distinguished from mildly affected shoots. The first signs of boron deficiency in the latter can be detected in midsummer, when the terminal bud and tip

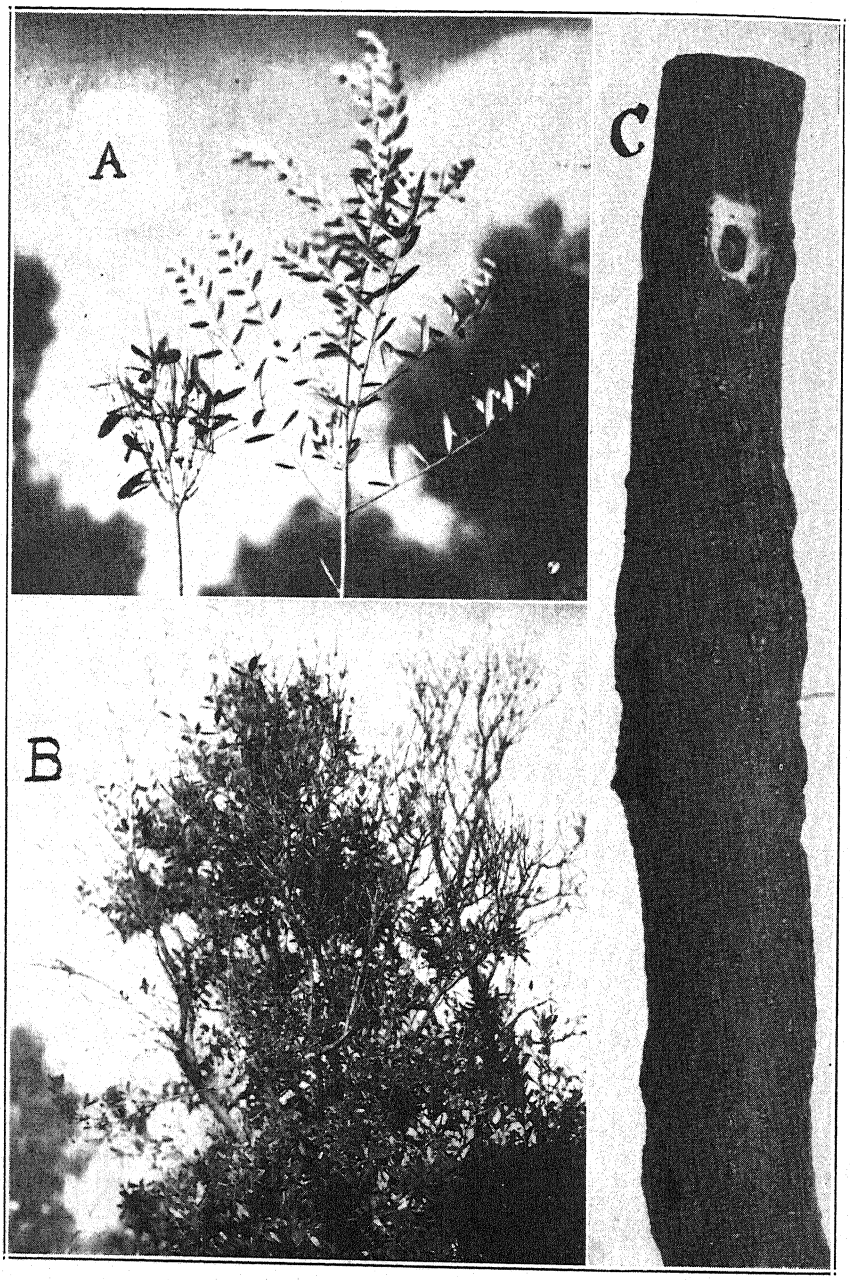



FIG. 2. A. At left a branch severely affected by boron deficiency; at right a normal branch (younger). B. Upper portion of a severely affected small olive tree. C. A branch with proturberances and internal necrosis of bark, ×1.

of the shoot die, usually involving one pair of immature leaves. Two or more secondary shoots are then produced from lower nodes. In severe cases the tips of these secondary shoots die in the same season.

Late in the fall, branches up to an inch or more in diameter die, with leaves persisting. Severe branch dieback has been observed on trees that had been growing fairly well a few weeks earlier and showing but mild leaf and shoot symptoms. Frequently, these branches that die appear to be the most vigorous in the tree. Some trees in the more severely affected areas in the orchards are eventually reduced to mere snags. A branch dieback of unknown cause exists in other olive districts, where boron deficiency is not suspected.

### Bark Symptoms

In some of the more severely affected trees the bark of larger branches produces smooth protuberances to perhaps 2 mm. above the level of surrounding bark and mostly 5 to 10 mm. long (Fig. 2, C). The bark underlying these protuberances contains varying amounts of brown necrotic tissue. On the same branches or elsewhere the bark may crack and become considerably roughened. As in the case of boron-deficient apples, however, the bark symptoms are not consistently associated with those of fruit and leaves.

## METHODS

The dry-salt method was employed in all limb injections of copper sulphate, manganese sulphate, zinc oxide, and boric acid. The powdered boric acid was mixed with an equal weight of diatomaceous earth (Celite) to facilitate measuring and handling.

A "gun" having the same dimensions as that described by Bennett (3) was used as a convenient instrument for measuring and inserting the dry salts. The boric acid-Celite mixture contained approximately $\frac{1}{4}$ g. of boric acid per shot. Dosages ranged from $\frac{1}{2}$ to 2 shots to each hole. One hole was used in branches 2 to 3″ in diameter and usually two holes in branches 4 to 6″ in diameter. The holes were 2″ or more in depth, bored with a 15/32-inch wood drill and plugged with $\frac{1}{2}$-inch hardwood dowels. Bark injury was reduced by dipping the ends of the dowels in soft wax before inserting.

Borax was applied with an orchard sprayer at 400 lb. pressure. A proprietary detergent (Dreft) was added to the June sprays to facilitate leaf wetting. The upper surfaces were wetted satisfactorily but the deposit on the under surfaces was scanty.

In the only soil treatment made prior to the summer of 1942 borax was spread by hand in bands around the trees, extending approximately from 2 to 10 feet from the trunk. The soil was not cultivated for several months after the application. About 10 inches of rain fell between the time of application and the summer dry season of 1942. Later soil treatments were made broadcast in irrigation furrows and in one case in solution applied from a spray tank.

RESULTS

Branch injections of boric acid, copper sulphate, manganese sulphate, and zinc oxide were made in Mission olive trees in 2 orchards in February, 1941. The trees were selected chiefly on the basis of tip dieback as described above. By July each of the 4 branches treated with boric acid showed definite improvement in growth when compared with the balance of the tree. From midsummer on, most of the terminal buds on nontreated portions of these trees died, while treated branches continued to grow vigorously. Two of the 4 branches treated with boric acid bore a heavy crop, while there was very little fruit on the nontreated branches. These treated branches also continued to grow normally throughout the second season. Additional branch treatments were made with copper, zinc, boron, and magnesium in November, 1941. No certain effect from any of these treatments could be seen in mid-June, 1942. By August, however, all of the boron-treated branches showed recovery and contrasted strikingly with the untreated portions and with branches treated with the other elements.

Five trees showing good vegetative growth but a high incidence of malformed fruit were treated in November, 1941. Each of the 5 trees was injected with boron, copper, manganese, and zinc, each in separate branches. In these trees the boron-treated branches bore more fruit in 1942 than the total produced on the balance of the tree. In addition to the increase in crop there was a sharp reduction in the amount of defective fruit on the boric acid-treated limbs (Table 1). No clear effect of copper, manganese, or zinc has been seen up to February, 1943.

TABLE 1.—*Effect of branch treatments on defective fruit of Mission olive. Hays Orchard, Butte County. Treated: November, 1941. Counts made: October, 1942*

| | Branch treatment | | | | |
|---|---|---|---|---|---|
| | None | Boron | Copper | Manganese | Zinc |
| Total fruit counted | 446 | 700.0 | 200 | 100 | 169 |
| Defective fruit | 174 | 17.0 | 70 | 27 | 64 |
| Percentage defective fruit | 39 | 2.4 | 35 | 27 | 37 |

Although boron seems to move laterally and downward more readily than some of the other minor elements, such movement is definitely limited. Thus, when branches 4 to 6 inches in diameter are injected in a single hole, the benefit is greater on that side of the branch than on the opposite side, at least so in the first season after treatment. Branches a foot below and in line with the point of injection are improved during the first season but not completely cured.

## Sprays

Mission olive trees were sprayed in March, 1942, with borax, 2 and 6 lb. per 100 gal.; manganese sulphate, 10 lb. per 100 gal.; 8–4–50 Bordeaux mixture and red copper oxide, 3 lb. per 100 gal. Some benefit was apparent

in growth, set, and quality of fruit on the trees sprayed with the 2 lb. dosage of borax, but the improvement was much more certain with the higher dosage. Copper and manganese were not effective. The March sprays were subjected to about 10″ of rain.

Additional spray applications were made on June 11, when most of the trees had just completed the blossom stage, but a few branches were still in full bloom. No rain fell during June, July, and August. The crop was markedly increased on most of the trees sprayed at this time in contrast to trees in nonsprayed rows, which continued to drop fruit throughout the season. Four trees severely stunted by boron deficiency, included in the 8 lb. to 100 gallon plot, failed to set any fruit, and were not greatly benefited in growth. Fruit counts on 10 nonsprayed trees in this orchard gave an average of 13.2 per cent defective fruit. Twenty-seven trees sprayed with 2, 4, or 8 lb. of borax to 100 gallons in March or June produced a much larger crop and only 0.41 per cent defective fruit. No significant difference was detected between trees sprayed in March or June.

June spray tests in a second orchard afforded less definite results, perhaps because of a mixture of varieties. In the same orchard one side of each of 6 severely affected Mission olives was sprayed with borax 8 lb. to 100 gallons on June 11. Improved twig growth was noted in July on the sprayed sides, but the benefit had disappeared by October. In a third orchard trees sprayed on June 11 seemed improved throughout the season.

## Soil Treatments

In February, 1942, Mr. Everett applied borax on the soil around 11 large trees in amounts ranging from 3 to 32 ounces per tree. All of these trees showed benefit in shoot growth by midsummer and good shoot growth continued through the season on trees that received 6 ounces or more. Some defective fruit (0.3 per cent to 9 per cent) was found at the 6-ounce dosage, but only one branch bore such fruit on the 4 trees that received 13 ounces or more. The 1942 crop was increased very considerably by the heavier dosages.

Borax treatments of ½ to 4 pounds per tree were made in 2 orchards in August, 1942. The material was lightly covered with soil in the bottom of the irrigation furrow shortly before the application of water. Clear benefit to shoots and leaves was apparent in one of these orchards in February, 1943. No evidence of boron toxicity to olive trees has been seen thus far in any of the soil treatments.

## Boron Deficiency in Other Crops in California

Unthrifty orange trees adjoining affected olive trees have been under observation in Butte County. Definite signs of boron deficiency were not detected in these trees, although the fruit seemed unnaturally hard and was subject to abnormal cracking at the blossom end. In June, four orange trees were irrigated in small basins with a solution of borax, supplying about ½ lb.

to each. Symptoms of excess boron developed in the leaves by August and were more evident in October. New growth was appearing at this time, but there was no clear benefit to growth as compared with that of neighboring trees during the current season. Young peach trees adjoining those of citrus and olive were not visibly benefited by a borax spray (2 lb.–100 gal.) applied in June.

Borax heretofore has been used in California as a soil amendment to a very limited extent, although deficiency symptoms in widely separated areas have been reported. Ark and Thomas (1) found that heavy soil applications benefited apple trees in Sonoma County. Corky fruit was found in one apple orchard in Eldorado County, and was corrected by trunk injections by Thomas in 1938. Larmer (8) described symptoms believed to indicate boron deficiency in sugar beets in the Salinas Valley but no field tests seem to have been made. C. M. Tompkins (unpublished) has seen boron deficiency symptoms in celery, radish, lettuce, and table beets in light, well-drained soils at Colma in San Mateo County and nearby in San Francisco County. Borax, broadcast at the rate of 20 lb. per acre and disked in just prior to seeding, eliminated symptoms in subsequent crops. O. A. Lorenz (unpublished) has observed the boron deficiency type of symptoms in table beets in Alameda, Monterey, Sonoma and San Joaquin Counties and has verified his diagnosis by pot cultures for the Sonoma soil. John T. Middleton (unpublished) observed symptoms attributed to lack of boron, on broccoli, cabbage, cauliflower, and rutabaga at San Juan Capistrano in 1940 and 1941. The symptoms usually were associated with limestone outcroppings where the soil pH was higher than in surrounding soil where plants were normal. Borax alone, applied for two seasons, failed to correct the symptoms; but, after sulphur treatment in the third season the crops were normal in these areas.

Ark and Tompkins (2) found that a borax solution eliminated symptoms in greenhouse Gloxinias. The symptoms developed with Gloxinias grown in "ratsnest," a mixture of forest débris collected in Marin and other coastal counties.

Trunk injections of boric acid made by the writers seemed to benefit Vinifera grapes in one Sonoma County vineyard. Low boron in the soil is indicated by the presence of pronounced leaf symptoms in olive near the vineyard. Attention was called to this vineyard because of defoliation and fruit shrivelling or failure to mature in 1941. The work on grapes has not progressed far enough to clearly define local symptoms, but those observed agree fairly well with the symptoms reported elsewhere (4, 10).

## DISCUSSION

The severely affected olive trees that attracted the particular attention of growers usually were in small groups in association with impaired drainage or excessive seepage of ground or irrigation water. With the discovery of boron deficiency and the leaf and fruit symptoms it became evident that the trouble was more extensive in the district than previously realized. The

tests to date have been confined to trees showing specific leaf or fruit symptoms or both. Extensive use of borax by olive growers during the winter of 1942–43 should show whether trees that do not exhibit the described symptoms will respond in growth or fruit production.

The olive is notoriously irregular in yield from season to season, tree to tree, and even from branch to branch in the same tree. However, a striking increase in crop from some of the treatments was obvious at a glance. Indeed considerable difficulty was often experienced in finding enough fruit on nontreated trees or branches to permit counts of percentages defective. Rather extensive yield records will be required nevertheless to determine the magnitude of crop increase.

The striking symptoms on the olive, some of which persist the year round, and the widespread planting of olive trees at roadsides and in gardens suggests that this tree may become a useful indicator of boron deficiency in areas not devoted to the commercial production of olives. Preliminary surveys seem to bear out this suggestion.

As to boron injury, Eaton (4) places the olive about midway in the semitolerant group. With the possible exception of one limb injection, no injury has been observed from any of the tests made by the writers. Soil application of a maximum of 8 lb. per tree are under way. Since large olive trees are improved by as little as 3 and 6 oz. of borax per tree and orange trees injured by 8 oz. per tree, it is obvious that the fixing power of the Aiken soil for boron is not high.

On the basis of the limited trials with olives and of experience elsewhere with deciduous fruit trees, commercial treatments have generally been at the rate of 1 lb. per tree or 50 lb. per acre. In some cases this dosage has been doubled in either a single application or by a second application on more severely affected areas. Fifty tons of borax for application during the winter of 1942–43 has been delivered to the growers of the Wyandotte district.

## SUMMARY

Characteristic pitting of fruits, chlorosis of leaf tips, and dieback of shoots and branches of the olive were reduced or eliminated by treatment with borax or boric acid. Branch injections, soil treatment, and spraying with boron compounds were all beneficial; but the effect of spraying on severely affected trees was transitory. Large trees responded to less than ½ lb. of borax broadcast on the soil in one district of Butte County, but seem to require about 1 lb. for complete cure. Four pounds per tree in the same soil did not cause discernible injury up to 6 months from the time of application.

Observations and tests of boron deficiency in other crops in California are summarized.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA.

LITERATURE CITED

1. ARK, P. A., and H. EARL THOMAS. Apple dieback in California. Phytopath. 30: 148–154. 1940.
2. ————, and C. M. TOMPKINS. The boron deficiency disease of Gloxinia and its control. Phytopath. 21: 467–469. 1941.
3. BENNETT, J. P. The treatment of lime-induced chlorosis with iron salts. California Agr. Exp. Stat. Circ. 321. 1931.
4. EATON, FRANK M. Boron in soils and irrigation waters and its effect on plants with particular reference to the San Joaquin Valley of California. U. S. Dept. Agr. Tech. Bull. 448. 1935.
5. ————, ROY D. MCCALLUM, and MILES S. MAYHUGH. Quality of irrigation waters of the Hollister Area of California with special reference to boron content and its effect on apricots and prunes. U. S. Dept. Agr. Tech. Bull. 746. 1941.
6. HORNE, WILLIAM T. Some diseases of the olive in California. The Olive Journal 1: 4, 10. 1917.
7. KELLEY, W. P., and S. M. BROWN. Boron in the soils and irrigation waters of southern California and its relation to citrus and walnut culture. Hilgardia 3: 445–458. 1928.
8. LARMER, F. G. Boron deficiency disease of sugar beets in the Salinas Valley of California. Sugar Beet Bull. 1: 4–6. 1937.
9. SCOFIELD, CARL S., and L. V. WILCOX. Boron in irrigation waters. U. S. Dept. Agr. Tech. Bull. 264. 1931.
10. SCOTT, L. E. An instance of boron deficiency in the grape under field conditions. Proc. Amer. Soc. Hort. Sci. 38: 375–378. 1941.
11. SMITH, R. E., and HAROLD E. THOMAS. Copper sulphate as a remedy for exanthema in prunes, apples, pears and olives. Phytopath. 18: 449–454. 1928.
12. WHETSTONE, RICHARD R., WILLIAM O. ROBINSON, and HORACE G. BYERS. Boron distribution in soils and related data. U. S. Dept. Agr. Tech. Bull. 797. 1942.

# THE INHERITANCE OF A WHITE MUTANT CHARACTER IN USTILAGO ZEAE[1]

E. C. Stakman, M. F. Kernkamp, W. J. Martin, and T. H. King

(Accepted for publication March 8, 1943)

Since Bauch[2] first reported the occurrence of sector variants in cultures of haploid lines of *Ustilago bromivora* (Tul.) Fish. v. Waldh., the phenomenon has been investigated extensively in several smut fungi, especially *U. zeae* (Beck.) Ung. Several investigators have shown that sector variants appear commonly in cultures of monosporidial lines of *U. zeae*. Stakman, Christensen, *et al.*[3] made an extensive study of variation in this fungus. They pointed out that numerous variants were produced in some monosporidial haploid lines but not in others, and that the tendency to sector could be increased in certain lines by certain environmental conditions. There were some indications from their investigations that factors for variant characters were inherited when mutants were crossed with other lines. All of the evidence available indicated that variants resulted from genetic changes and probably could not be attributed to delayed segregation but rather to mutation.

Stakman, Tyler, and Hafstad[4] studied the constancy of cultural characters of a number of variants of *Ustilago zeae* and confirmed the previously expressed opinion that variant lines retained their distinctive characters, although subject to pronounced phenotypic variability. In addition, they studied the pathogenicity of a haploid monosporidial line and several of its variant derivatives by injecting them into corn, each in separate combinations with two haploid monosporidial lines of different sex. They concluded that variants in general tended to be less virulent than their parents, but that an occasional variant might be somewhat more pathogenic. As the resulting dikaryophytes differed distinctly in pathogenicity, it was evident that the variants were different genetically from the parental lines. Furthermore, the pathogenicity of the various combinations was the same in tests made about $3\frac{1}{2}$ years apart, thus again showing persistence of variant characters. The conclusion regarding genotypic differences between the parental line and its variants was based on relative behavior in the dikaryo-

[1] Paper No. 2073 of the Journal Series of the Minnesota Agricultural Experiment Station. Supported in part by a grant from the research funds of the Graduate School of the University of Minnesota.

Assistance in the preparation of these materials was furnished by the personnel of the Works Projects Administration, Official Project No. 65-1-71-140, Subproject No. 491, Sponsored by the University of Minnesota, 1941.

[2] Bauch, R. Untersuchungen über die Entwickelungsgeschichte und Sexual-physiologie der *Ustilago bromivora* and *Ustilago grandis*. Ztschr. Bot. 17: 129–177. 1925.

[3] Stakman, E. C., J. J. Christensen, C. J. Eide, and Bjorn Peterson. Mutation and hybridization in *Ustilago zeae*. Minn. Agr. Exp. Stat. Bull. 65. 1929.

[4] Stakman, E. C., L. J. Tyler, and G. E. Hafstad. The constancy of cultural characters and pathogenicity in variant lines of *Ustilago zeae*. Bull. Torr. Bot. Club 60: 565–572. 1933.

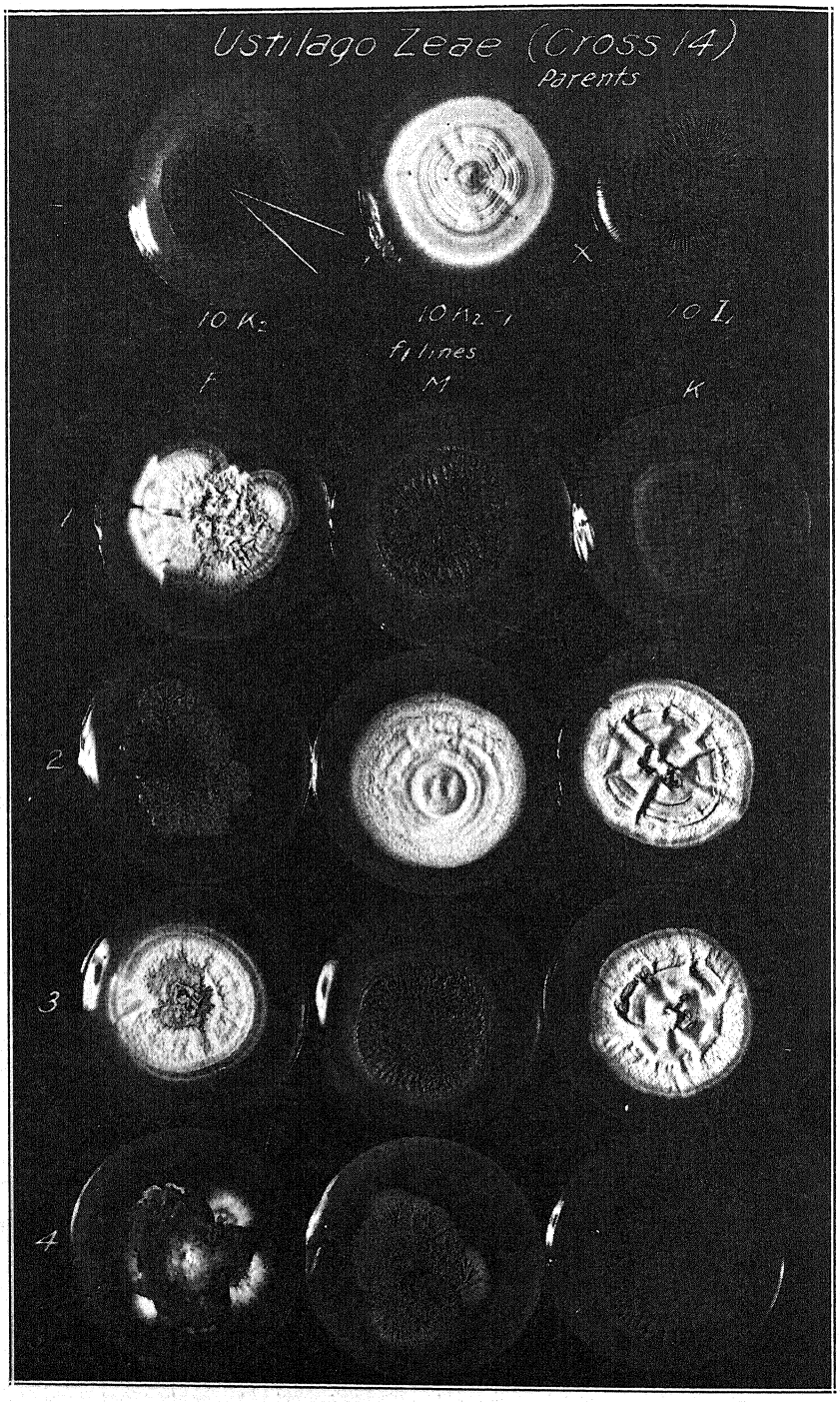



FIG. 1. Origin of mutant and inheritance of mutant characters. 10K2 is the brown line, characterized by radial folds, from which the white mutant, with concentric rings, 10K2–1, arose. Colonies from the four sporidia from each of three chlamydospores, F,

phase; but it was not shown that the variant characters were transmitted to $f_1$ segregates, the haploid progeny of crosses between haploid lines. Therefore, further work was done along this line, and conclusive evidence was obtained that factors for variant characters are inherited. Stakman[5] presented evidence that factors for mutability are inherited just as are factors for cultural characters.

During the course of extensive experiments on variation, according to evidence presented briefly by Stakman *et al.*,[3] it was found that delayed segregation could scarcely account for the production of sector variants. It was quite clear that a culture derived from a single sporidium constituted a pure line, barring subsequent mutation.

Attempts were made to find a mutant character so distinct as to enable the easiest possible recognition in segregates from crosses. This seemed particularly important because there is such extensive recombination in many crosses that even the parental types sometimes are not recovered among the $f_1$ segregates. Several years ago a mutant of the desired type appeared, and it has been studied since that time. As only some of the results are pertinent to the present discussion, a relatively brief summary is presented here. Further details are given in a paper on the inheritance of factors for constancy and for variability in *Ustilago zeae*.[6]

In 1934, an apparently pure white sector (designated 10K2–1) appeared in a colony of 10K2, a monosporidial line that always produced brownish colonies tinged with vinaceous. This white sector contrasted so strongly with the colony as a whole that transfers were made from it, and the resulting cultures were compared in detail with the parent. It was evident that most of the factors for pigmentation had been lost as there was only a tinge of color in the mutant under some conditions, whereas the parent culture in which the mutant arose was always brownish. In addition to loss of factors for pigmentation, the mutant had a tendency to produce abundant aerial conidia, which accentuated the impression of whiteness. In this respect the mutant also differed decidedly from the parent 10K2.

The mutant differed from its parent in a third character, namely, the tendency to produce numerous narrow, concentric rings, whereas the parental line had a tendency to produce more or less distinct radial furrows (Fig. 1). It was evident, therefore, that the mutant, designated 10K2–1, differed from its parent 10K2 in three distinct characters. Both the parent and the mutant were then crossed with a monosporidial line 10I1. This line is shown in figure 1, and a brief description of it is given in table 1. All three mutant characters appeared in various combinations in the $f_1$

[5] Stakman, E. C. The problem of specialization and variation in phytopathogenic fungi. Genetica 18: 372–389. 1936.

[6] Stakman, E. C., M. F. Kernkamp, T. H. King, and W. J. Martin. Genetic factors for mutability and mutant characters in *Ustilago Zeae*. American Journal of Botany 30: 37–48. 1943.

M, and K, resulting from the cross between 10K2–1 and 10I1 are shown below. The white color of the mutant appears in the segregates from each clamydospore. Of the five white lines, three (F1, F3, and K3) are sectoring.

progeny of the cross of 10K2–1 × 10I1. There were no white segregates, and none of the other mutant characters appeared in the progeny of the cross of the 10K2 (parent) × 10I1.

TABLE 1.—*Cultural characters of monosporidial lines 10I1, 10K2, and mutant from 10K2, 10K2–1.*

| Lines | Color | Topography | Margin |
|---|---|---|---|
| 10I1 | Clear brown to tan, tinged with purple | Close pronounced radial folds tapering toward periphery | Clear, somewhat ciliate |
| 10K2 | Warm buff to brown, with vinaceous tinge | Fine radial folds; confused clockwise folds in center; counter-clockwise folds towards margin | Clear, somewhat ciliate |
| 10K2–1 | White | Umbonate; with distinct concentric ridges | Silky, ciliate |

For the sake of clearness, only the inheritance of factors for "white" color is emphasized in this paper. It should be pointed out that there are varying degrees of whiteness, but segregates were recovered that were as white as the mutant. The impression of whiteness is given by the relative absence of pigment in the compact mycelial growth of colonies plus the rather abundant development of aerial conidia on the surface. Figure 1 shows single colonies of 3 sets of sporidia isolated from 3 promycelia from chlamydospores resulting from crossing the mutant 10K2–1 with 10I1. It will be noticed that 5 of the segregates are distinctly white in appearance, but none of them is exactly like the mutant parent and only 2 of them are alike. There had, therefore, been a certain amount of recombination, but the most conspicuous feature common to all of them is their whiteness. It will be observed also from figure 1 that three of the white segregates, F1, F3, and K3, are producing sectors different in appearance from the rest of the colony. Many such sectors were studied and proved to be distinct.

From cross 14 (10K2–1 × 10I1), 10 out of 38 $f_1$ segregates were white. The same cross was made again and 9 out of 28 segregates were white, making a total for this cross of 19 white segregates out of 66 that were studied.

One of the white segregates from cross 14, designated 14J4, was then crossed with 26G1–2, a line that was as nearly black in culture as any that the writers have studied. This cross was designated cross 300. Of 32 segregates from this cross, 18 were white or near white; single colonies of sets of four sporidia from the promycelia of each of three chlamydospores are shown in figure 2.

Several other white lines from cross 14 were crossed with other dark-colored lines, and in every case white segregates appeared.

At present there are 83 distinct white or near white derivatives from the original white mutant, all of which arose as segregates from crosses involving progeny of the original mutant.

As it was apparent that the white mutant character was due to genetic

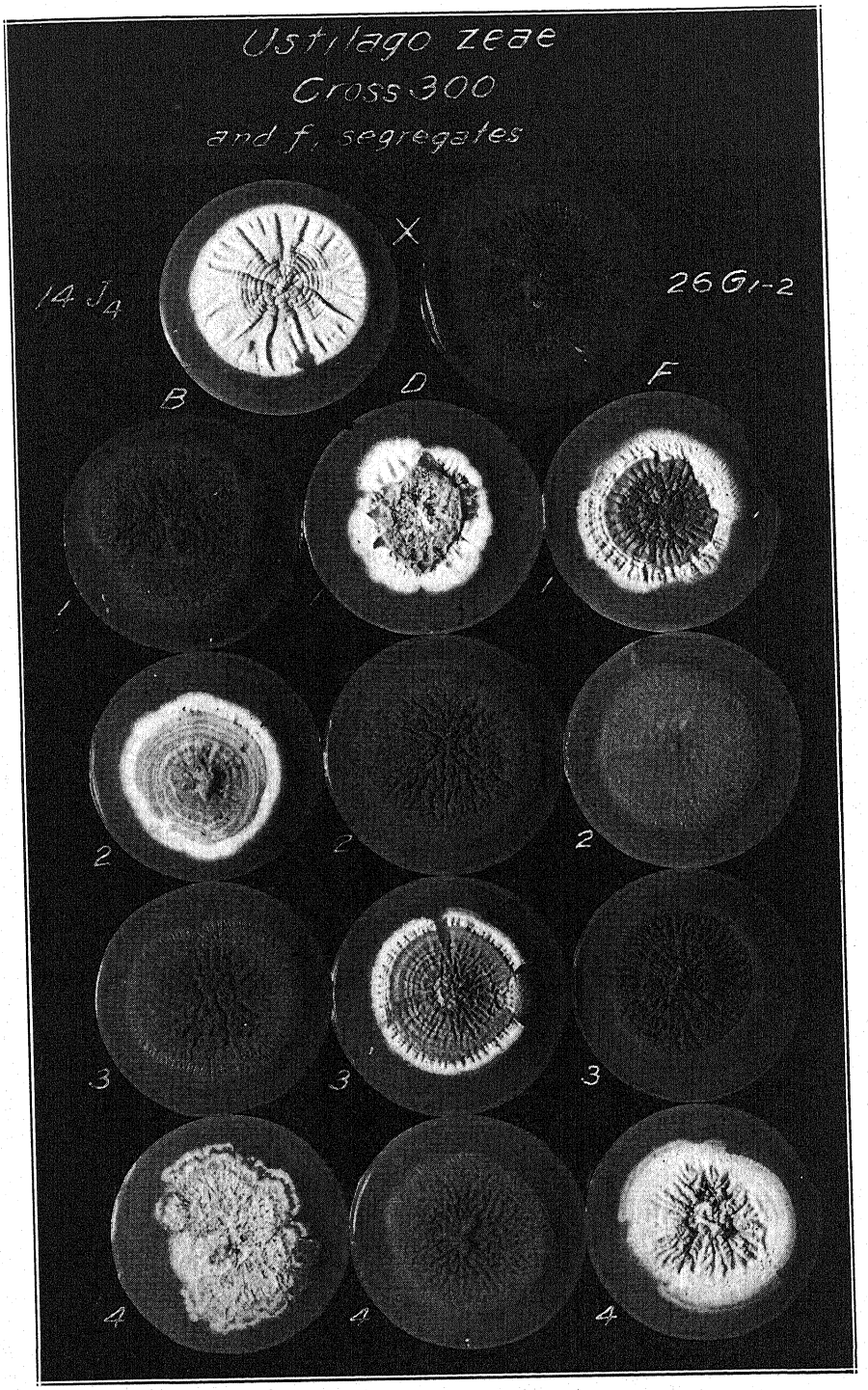



FIG. 2. Single colonies from the four sporidia of the promycelia of three chlamydospores, B, D, and F, from the cross between the white line, 14J4, and the black line, 26G1–2. The white color appears among the segregates from each chlamydospore.

factors that were inherited in crosses, there can be no question that genetic changes were involved and that the genes for this character were inherited in the same way as those for other characters. It seemed possible, therefore, that a white smut might be produced by appropriate crossings of white × white.

Thirty-nine of the whitest lines available were mated in various combinations, making a total of 417 matings, and inoculated into corn seedlings, both in the greenhouse and in the field. Many of the compatible combinations produced large galls on the corn plants, indicating that the compatible lines were of opposite sex and contained the necessary factors for pathogenicity and gall formation. Surprisingly enough, however, there were no mature chlamydospores in any of the galls and only in a few galls were there apparently immature chlamydospores. After repeated attempts to obtain combinations that would produce chlamydospores, all of the white lines were mixed together and inoculated into corn. Large galls were formed, but there never were any mature chlamydospores. These trials were repeated several times, but the results were always the same.

It appears, therefore, that the white mutant had lost certain factors for ability to fuse with lines of its own kind. The necessary factors for nuclear association and the production of the dikaryophase are present; furthermore, the necessary factors for pathogenicity obviously are present in many of the dikaryophytes, since they produced normally and, in some cases, even extraordinarily large galls. Nevertheless, the necessary factors for nuclear fusion and the production of zygotes, the chlamydospores, apparently are lacking. That these white lines are not all of the same sex is indicated by the fact that only certain combinations among them will produce galls while others will not, and furthermore by the fact that when white lines are crossed with a random sample of tester lines, they assort themselves into various sex groups, which produce normal chlamydospores as is true of a random sample of lines in general.

Finally, corn was inoculated with a mixture of 12 white monosporidial lines derived from haploid segregates from 5 different crosses. Galls were formed but they contained no chlamydospores nor chlamydospore-like bodies. Material from some of these galls was killed in formal-acetic alcohol, run through the butyl-alcohol series, embedded, cut about 8 microns thick, and stained by the Feulgen method. The mycelium was as clearly dicaryotic as in normal galls. Hence it appears that certain combinations of the white lines under consideration produce a normal dicaryophase, without being able to produce the diplophase.

## SUMMARY AND CONCLUSIONS

From the foregoing it is evident that factors for the white mutant characters that arose in a brown colony are heritable through several successive sexual generations. This should furnish final proof that such sector variants are mutants. That a genetic change had taken place is perfectly

obvious, and that the new characters, are persistent both in asexual propagation and sexual reproduction is also clear. One of the most significant facts, however, is that it never has been possible to obtain functional chlamydospores from crosses between the white derivatives of the original white mutant. In a sense this indicates that there are multiple factors for sex. The nuclei of certain white lines have the necessary factors for attraction and association that enable them to produce the dikaryophase, but they seem to lack the necessary factors for complete sexual fusion. This experience and many others of similar nature suggest strongly that there are gradations in the degree of maleness or femaleness, if these terms could be applied to an organism that is completely isogamous as far as can be observed. It is interesting also to note that factors for pathogenicity are apparently different from those for sex. It is true that *Ustilago zeae* is pathogenic only in the dikaryophase, or, in exceptional cases, in the diplophase; consequently, the association of nuclei of two kinds is prerequisite to parasitism in the corn plant. But these two nuclei are not necessarily able to fuse and produce functional zygotes or chlamydospores.

MINNESOTA AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY FARM, ST. PAUL, MINNESOTA

# THE NATURE OF ULTRA-VIRUSES AND THEIR BIOLOGICAL ACTIVITY[1]

V. L. Rischkov

(Accepted for publication January 16, 1943)

Half a century ago D. I. Ivanovsky (5) discovered ultra-viruses. At the dawn of virulogical investigations two points of view concerning the nature of viruses were advanced. According to the conception of Beyerinck (2), ultra-viruses differ from microbes in principle (*Contagium vivum fluidum*); according to Loeffler and Frosch (6), they are simply very minute microbes. The isolation by Stanley (16) of the purified virus of tobacco mosaic showed that the direction of virulogical research pointed out by Beyerinck is a more fruitful one. Thanks to the discovery of virus nucleoproteids and their subsequent physico-chemical analysis, a magnificent picture opened before our eyes. Biological individuals are to be found in the world of molecules, subject to the laws of microphysics. From a nude protein molecule to a real microbe there exists a whole series of intergradations. This series includes elementary bodies (about which the discussion still lasts whether they are molecules or protoplasts separated from the surrounding world by semipermeable membranes), real ultra-microbes leading the life of parasites, and saprophytic ultra-microbes. At present we do not know whether this series is an ascending or descending one. It is also certain that to a definite degree it is an artificial series. Nevertheless, it obliterates the border line between molecules and protoplasts, a most remarkable regularity is observed in this series, which can be formulated thus: the simplification of the organization of the cell and its conversion into precellular condition is connected with the concentration of nucleoproteids. The cells of bacteria are richer in nucleoproteids than those of higher organisms, elementary bodies are richer in nucleoproteids than the cells of bacteria, viruses pathogenic for plants are pure nucleoproteids. The statement that nucleoproteids form the substratum of continuity and variability of the most elementary biological units is firmly established through virulogical investigations.

Up to recent times viruses were studied chiefly from the physical and chemical points of view. Notwithstanding the importance of these studies, they cannot answer the most fundamental questions concerning the mechanism of autoreproduction of virus molecules and the mechanism of their action. To the study of these questions the works of the writer and his collaborators have been devoted.[2] Though the results obtained are far from giving an exhaustive answer to these questions, they nevertheless include a certain program of research, and plan concrete ways of experimental approach to these difficult questions.

[1] Report made at the Session of the Academy of Sciences of the Ukrainian S. S. R. (12th–17th Jan., 1942).

[2] Institute of Microbiology, Academy of Sciences of the U. S. S. R.

At the beginning of our investigations we made an attempt to find out what the virus takes from the plant by means of fractioning the proteins of the diseased plant. We failed to detect a deficit in the protein fraction, which coagulates at 70° C. in the juice of tomatoes affected by mosaic disease; on the contrary, we got the impression that, alongside the virus protein in the diseased plant, there can accumulate an inert non-virus protein—a phenomenon already pointed out by other authors (11).

If diseased and normal plants are grown under conditions of phosphorus and nitrogen starvation, the diseased plants lose less protein than the normal ones and, consequently, the difference in the contents of protein nitrogen between diseased and normal plants is more pronounced in starving plants than in those that obtain normal nutrition (Table 1).

TABLE 1.—*Contents of protein N in leaves of tobacco under different nutrition (in per cent of dry weight)*

| Nutrition | Normal | Diseased | In per cent of control |
|---|---|---|---|
| NPK | 2.06 | 3.61 | 172.9 |
| 0.25N/PK | 1.05 | 2.54 | 240.5 |
| N/0.25 P/K | 1.62 | 3.44 | 212.8 |

Some literature data (4), as well as other works of the writer, completely affirm our data concerning a more economical utilization of protein by plants affected by virus diseases.

The investigation of nitrogen metabolism does not indicate what is taken from the plant by the virus; an exception is found only in the data of our collaborator, P. A. Agatov (1), who showed that the proteins of tomatoes affected by the mosaic disease contain somewhat less argynine and proline than the proteins of a normal plant. Failing to find in the nitrogen metabolism an answer to the question investigated, we turned to the study of phosphorus metabolism in case of virus diseases. It was found that tobacco infected by the mosaic disease contains no more (and often less) phosphorus than a normal plant. The lipoid phosphorus undergoes no significant changes, whereas the changes in protein phosphorus are rather clearly manifest. The amount of phosphorus in soluble proteins in case of mosaic disease increases, the amount of phosphorus in insoluble structural protein de-

TABLE 2.—*Forms of P in leaves of mosaic-diseased and normal tobacco (in percentage $P_2O_5$ of dry weight)*

| Material | Total | Mineral P and phytine | Organic P | Protein-soluble P | Protein-insoluble P |
|---|---|---|---|---|---|
| Normal | 1.24 | 0.59 | 0.30 | 0.067 | 0.34 |
| Diseased | 1.18 | 0.54 | 0.34 | 0.198 | 0.28 |
| Normal | 1.23 | 0.51 | 0.28[a] | ..... | 0.44 |
| Diseased | 1.24 | 0.57 | 0.39[a] | ..... | 0.40 |

[a] Including protein soluble.

creases. It seems that in case of virus diseases there takes place an expenditure of structural nucleoproteids (Tables 2 and 3).

TABLE 3.—*Forms of phosphorus in leaves of mosaic-diseased and normal tobacco (in percentage dry weight)*

| Material | Total | Lipoid | Soluble in 0.1 N HCl | Insoluble |
|---|---|---|---|---|
| Normal | 2.25 | 0.19 | 1.54 | 0.62 |
| Diseased | 1.89 | 0.16 | 1.34 | 0.56 |

A comparison of normal and diseased plants is insufficient for a characteristic of biochemical changes that are connected with the accumulation of the virus, because the picture is evidently strongly marred by diverse secondary factors. In order to eliminate as far as possible these secondary factors we compared leaves rubbed with the juice of a normal tobacco plant with those rubbed with the juice of a tobacco plant infected by mosaic, or compared isolated halves of leaves likewise rubbed with normal juice or with juice containing the virus.

Thus, leaves accumulating and not accumulating the virus were compared. Externally, they differed but insignificantly, those accumulating the virus being on the average somewhat yellower than normal ones. The data presented in table 4 show that the accumulation of the virus is accompanied by an expenditure of carbohydrates; while normal leaf halves have spent a considerable amount of protein, leaf halves accumulating the virus have scarcely spent protein at all.

TABLE 4.—*Carbohydrates and nitrogen in normal tobacco leaves and in leaves accumulating the virus (in percentage dry weight)*

| Material | Glucose | Saccharose | Starch | Protein N | Total N |
|---|---|---|---|---|---|
| Leaves rubbed with normal juice | 0.85 | 0.71 | 1.50 | 3.89 | 5.93 |
| Leaves rubbed with infected juice | 0.49 | 0.28 | 0.26 | 4.07 | 6.05 |
| Isolated leaf halves rubbed with normal juice | 0.15 | 0.35 | 0.61 | 3.32 | 6.08 |
| Isolated leaf halves rubbed with infected juice | 0.11 | 0.23 | 0.04 | 4.03 | 5.76 |

We further sought in the possible biochemical activity of the virus an explanation of its powerful action on the metabolism of the plant. The first tests of the purified virus of tobacco mosaic in respect to its possible fermentative activity gave negative results (8). However, works in this direction were continued in our laboratory. M. N. Vorobiova (18) observed that the protein of the virus of tobacco mosaic, when activated by CN or HS, can hydrolize the tomato protein. In cooperation with this author we were able

to detect the ability of this virus to liberate phosphoric acid from monophosphate of glucose (Table 5).

These data as to the fermentative activity of filtrable viruses agree with some literature evidence, especially concerning bacteriophage and ele-

TABLE 5.—*Action of the phosphatase of the virus of tobacco mosaic disease*[a] *(pH 5.6, temperature 37° C., substratum-monophosphate of glucose)*

| Mineral P in percentage | | Percentage of control | Exposition in hours |
|---|---|---|---|
| Control | Experiment | | |
| 0.16 | 0.19 | 120.2 | 2 |
| 0.15 | 0.22 | 147.9 | 18 |
| 0.13 | 0.22 | 174.5 | 18 |
| 0.24 | 0.55 | 225.8 | 20 |
| 0.15 | 0.40 | 257.5 | 18 |

[a] Note: in all the experiments except the second one magnesium sulphate was added to obtain the activation of phosphatase.

mentary bodies of vaccine, but require further verification and expansion. Seemingly, viruses actually have the action of phosphatase and one of the primary links of their biochemical activity is formed by phosphorilizing of carbohydrates and therefore the activation of carbohydrate metabolism, as well as the disintegration of structural nucleoproteids of the protoplast infected by the virus.

We also sought the elucidation of the relation between the protoplasm and the virus in experiments concerning the influence of starvation on the titre of the virus. In these works fulfilled partly in co-operation with Smirnova (9), partly by Smirnova alone (13), we failed to detect a decrease of the titre of the virus in plants deprived of nitrogen or phosphorus. Spencer agrees with us that the virus protein, when once formed, is not destroyed in starving plants; however, this author thinks that in tobacco plants deprived of nitrogen the rate of accumulation of the virus is slackened. The discrepancy of our results probably depends in a large degree upon the fact that Spencer freezes the plants whence he draws the juice for the determination of the titre of the virus. This procedure is connected with a partial inactivation of the virus, and our preliminary data show that this inactivation is accelerated in plants deprived of nitrogen.

An investigation of the mechanism of the accumulation of the virus is impossible without a thorough knowledge of the physiological conditions necessary to such an accumulation. Strangely enough, data on this question are nearly absent in literature. Here we applied the leaf halves method. Tobacco leaves separated from the plant were rubbed with the juice containing the virus, which was subsequently thoroughly washed from the surface under a water tap. The leaves were cut in halves, each experimental half corresponding to a control one. The experimental halves were exposed to the action of different temperatures, were placed in an atmosphere of pure hydrogen or were infiltrated with different substances.

By this means we were able to ascertain that the accumulation of the virus of tobacco mosaic disease is already absent at a temperature of 5° C., and proceeds much more slowly at 15° C. than at 20° C. (Table 6). The temperature curve of the accumulation of the virus is to be given later on (14). The virus continues to accumulate in an atmosphere containing 0.5 cc. of

TABLE 6.—*The influence of temperature on the titre of the virus of tobacco mosaic disease*

| Temperature | Number of necroses | |
|---|---|---|
| | On the 4th day | On the 9th day |
| 15° C. | 16.5 | 58.6 |
| 20° C. | 55.0 | 89.4 |

ethyl ether per litre as intensively as without ether. In higher concentrations of ether the accumulation of the virus slackens, but here is observed a certain injury of the leaves. In an atmosphere of hydrogen the tobacco leaves suffer too much to allow the solving of the question whether the cessation of the accumulation of the virus is due to the deprivation of the leaves of oxygen or is a by-product of the poor condition of the cells (11). Experiments of Woods (19) analogous in method, give evidence that the accumulation of the virus depends on an oxidative system, reversibly inactivated by CN.

The above examples show the ways where we sought answers to the questions concerning the biological activity of the virus. Certainly these investigations should be accompanied by direct observations of the cell affected by virus disease and cannot be separated from the structural conditions of autoreproduction of the virus. The paracrystalline and crystalline condition of the virus in the protoplasm must play an important role in the mechanism of its accumulation (10). It is possible that in the future the applications of Cassperson's (3) method to a cell affected by virus disease will render a significant service for our deeper understanding of the relation between the protoplasm and the virus, four our further understanding of the mechanism of its action and autoreproduction—questions which, we believe, occupy a central position in contemporary biology.

## LITERATURE CITED

1. AGATOV, P. A. Biochimia 6: No. 1. 1940.
2. BEYERINCK. Zbl. Bakt. II, 5: 27. 1899.
3. CASSPERSON, T. Naturwiss. 28: Nos. 31–32. 1940.
4. CORDINGLY, H., I. GRAINGER, W. H. PEARSALL, and A. WRIGHT. Ann. Appl. Biol. 21: 78. 1934.
5. IVANOVSKY, D. Bull. de L'acad. imper. d. Sc. de Petersbourg 13: 237. 1892.
6. LOEFFLER und FROSCH. Deutsch. med. Wschr. 80. 1898.
7. RISCHKOV, V., and E. GROMYKO. Microbiology, No. 3. 1938.
8. ———, and K. SUKHOV. Compte-Rendu de l'acad. d. Sci. de l'URSS 21: No. 5. 1938.

9. ————————, and V. SMIRNOVA. Ebenda 23: No. 1. 1939.
10. ————————, and ————————. Ebenda. 1941.
11. ————————, and E. GROMYKO. Microbiology 10: Nos. 7–8. 1941.
12. ————————. Nature of the filtrable viruses and the problem of their "physiology." "Plant Virus Diseases and their Control." Transactions of the Conference on Plant Virus Diseases. (Moscow) 1941.
13. SMIRNOVA, V. A. Microbiology 9: Nos. 9–10. 1940.
14. ————————. Ebenda 10: Nos. 7–8. 1941.
15. SPENCER, E. Plant Physiology 16: No. 2. 1940.
16. STANLEY, W. Science 81: 644. 1935.
17. ————————. Phytopath. 27: 1152. 1937.
18. VOROBIEVA, M. Compt. Rend. l'Academie des Sci. de l'URSS 30: No. 5. 1941.
19. WOODS, M. W. Reversible inhibition of tobacco-mosaic virus in living cells with 0.0002 molar sodium cyanide. Science 91: 295–296. 1940.

# A NEW SPECIES OF FUSARIUM CAUSING VASCULAR WILT OF TOMATO

FREDERICK L. WELLMAN[1]

(Accepted for publication March 29, 1943)

The purpose of this note is to remind plant pathologists that a new species of *Fusarium,* causing vascular wilt of tomato, has been known for some time,[2] and to so describe it that other investigators may watch for and recognize it. The original isolate came from V. A. Wright, formerly of

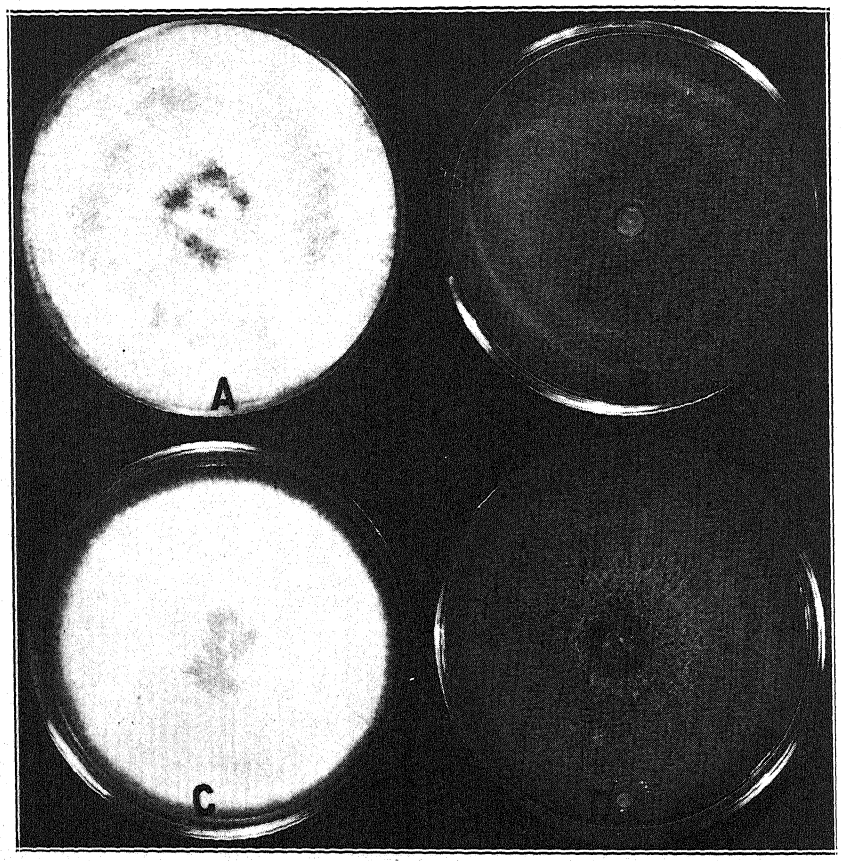



FIG. 1. Comparative growth in Petri-plate cultures of *Fusarium retusum* (A, raised type, note coarse aerial growth completely filling air space in dish; B, appressed type) and of *F. bulbigenum* var. *lycopersici* (C, raised type, fine, woolly; D, appressed type).

[1] Senior Agriculturist, Office of Foreign Agricultural Relations; formerly Plant Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Dept. of Agriculture, Bureau of Plant Industry Station, Beltsville, Maryland.

[2] Wellman, F. L., and Dorothy J. Blaisdell. U. S. Dept. Agr. Techn. Bull. 705. 1940.

the Department of Botany of the Indiana Agricultural Experiment Station, with the notation: "Isolated November 1937, from a fruit borne by a plant showing severe wilting. The vascular tissue in the fruit itself showed a darkening in color. The plant was grown in a wilt-infested field near Trafalgar, Indiana." Dr. Wright released the material to me for taxonomic treatment.

Bonny Best and Marglobe tomato plants were inoculated with the Trafalgar organism. Wilt symptoms resulted, identical with those caused by *Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and Rank. Comparative cultural and microscopic studies were made of these two organisms. A search through all available literature dealing with *Fusarium* disclosed no described species to which the Trafalgar organism could be assigned. Morphologically, it was widely divergent from *F. bulbigenum* var. *lycopersici*, and was, therefore, unquestionably different from the new form of the latter reported by Reinking.[3] It was found by cultural manipulations that selected isolates of the new species could be separated into the same cultural classes as have been reported[2] for the common tomato wilt *Fusarium*.

### TECHNICAL DESCRIPTION OF **Fusarium retusum** SP. NOV.

Growth on potato dextrose agar (Fig. 1) formed a slightly coarse, loose, and fluffy, rather "wild-growing" mat that, within a few days, filled the air space above the medium in the Petri dish or test tube. Colors[2] varied from white, livid-pink, and shades of yellow, through orange, tawny-olive, and garnet-brown, to Morocco red. A few sclerotia developed; at first white, becoming greenish. Cream-color sporodochia developed consistently in few monosporic isolations, but never in mass isolates. Reisolation and histological studies demonstrated infection hyphae growing only inside xylem elements of diseased tomato stems. With regard to morphology (Fig. 2) hyphae of fungus culture, hyaline, septate, branched, may be faintly tinged with yellow or red, and range from 2 μ to 7.5 μ in diameter; sporophores hyaline, bostrychoid, much branched, bearing easily detached microspores and macrospores; macroconidia, colorless and hyaline, straight to slightly curved, with a rounded, blunt apex and a distinct foot on the proximal end, 1–5-septate, mostly 3-septate, with average dimensions of about 26.8 μ long and about 4.3 μ wide; microconidia hyaline, colorless, mostly oblong and umbonate at the base, largely unicellular, rarely 1-septate, with average dimensions about 9.9 μ long and about 3.8 μ wide; chlamydospores may vary greatly in size and shape, are borne mostly in intercalary positions in mycelium or in macrospores, are colorless, and densely filled with protoplasmic granules. Natural occurrence in darkened vascular system in fruit of a severely wilted tomato plant near Trafalgar, Indiana.

Hyphae fertiles incoloratae, valde ramosae, bostrychoideae, et macroconidia et microconidia ferentes; macroconidiis hyalinis, incoloratis, rectis vel paulum curvatis, apice retusis, basi vulgo oblique pedicellatis, 1–5 septatis plerumque 3-septatis, saepius circa 26.8 μ longis et circa 4.3 μ crassus; microconidiis hyalinis, incoloratis, vulgo oblongatis et basi umbonatis, plerumque continuiis rarius uniseptatis, saepius circa 9.9 μ longis et circa 3.8 μ crassis. Chlamydosporae in mycelio etiam in macroconidiis ortae, plerumque intercalares, incoloratae, protoplasmatis granulosi repletae. Habitat in fructibus Lycopersici esculenti languidi prope Trafalgar, Indiana.

The well-known tomato organism, *Fusarium bulbigenum* var. *lycopersici*, is much slower in growth than *F. retusum*, and also varies from it in having much longer and more slender macrospores with quite pointed ends. My studies indicate that *F. retusum* is well outside the systematic limits of either the *Elegans* group[4,5] or the species *F. oxysporum*[6] with which the

[3] Reinking, O. A. Farm Research. New York (Geneva) 3: 12–13. 1937.
[4] Wollenweber, H. W. Fusaria Autographic Delineata. Berlin, 1930.

well-known organism is closely associated. It would appear from its cultural and morphological characters, that *F. retusum* may be considered a member of the systematic group *Sporotrichiella*[4,5] and possibly approaches nearest to the species *F. poae* (Pk.) Wr. It differs from this latter species,

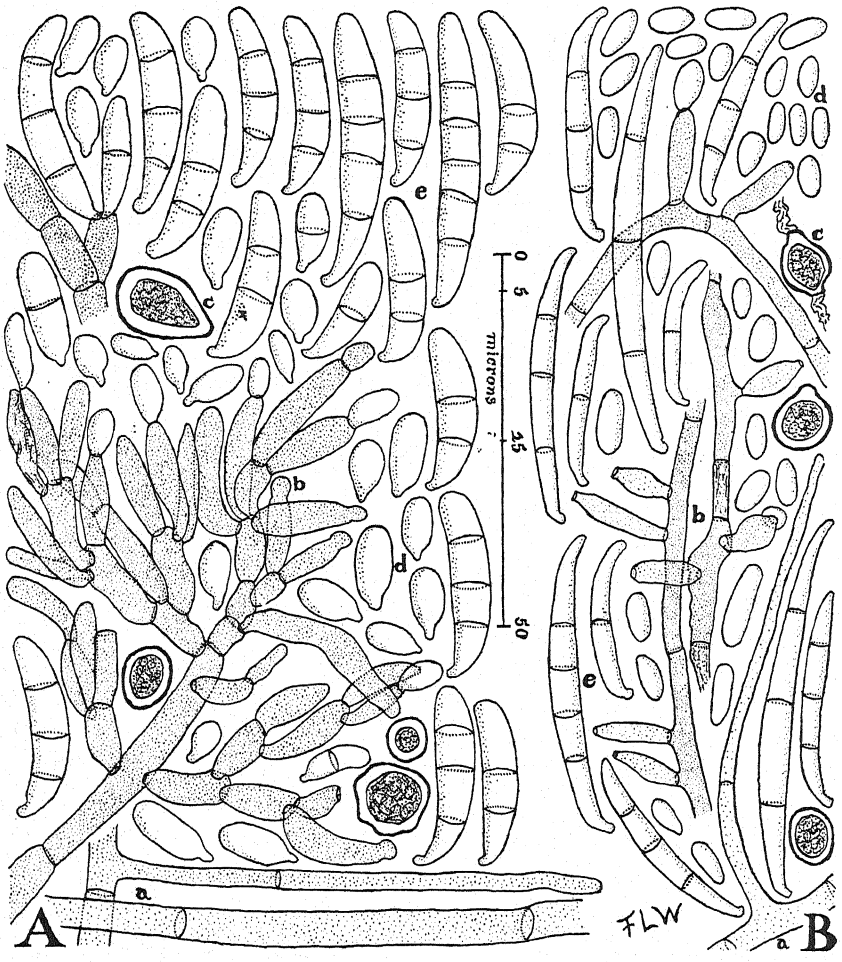



FIG. 2. Comparative morphology of (A) *Fusarium retusum* and (B) *F. bulbigenum* var. *lycopersici*. a, Mycelium; b, conidiophores; c, chlamydospores; d, microspores; e, macrospores. Magnification ×1000; drawings made with aid of camera lucida.

however, in the shape of its macrospores, whose blunt and rounded distal ends are very distinctive. The appellation *retusum*, which I have given to the new species, is from the Latin, meaning blunt. (I am indebted to Charles Drechsler for writing the Latin diagnosis.)

[5] Wollenweber, H. W., and O. A. Reinking. Die Fusarien. Berlin, 1935.
[6] Snyder, W. C., and H. N. Hansen. Amer. Jour. Bot. 27: 64–67. 1940.

# A BACTERIAL LEAF SPOT AND BLIGHT OF THE RUSSIAN DANDELION[1]

JOHN S. NIEDERHAUSER[2]

(Accepted for publication, July 27, 1943)

In the course of an investigation of diseases of the Russian dandelion, *Taraxacum kok-saghyz* Rod., a severe leaf spot and blight was noted. When first observed in early September, 1942, the disease was common to nearly 15 per cent of the plants in the test plot at Ithaca, New York, and it gradually became more severe until checked by a frost in mid-October. During this time there was much cloudy, wet weather.

The lesions occur on the leaves only, and vary in size from minute dots to large necrotic areas that may occupy nearly the entire blade. The diseased areas are uniformly black in color, with a narrow, yellow, chloranemic border. If there are large lesions near the base of the leaf, the distal portions may turn prematurely yellow. Ordinarily, the older, outer leaves are most severely affected.

Dilution plates made from bits of diseased leaf tissue constantly yielded a yellow bacterium that produced characteristic spreading, gummy, moist-shining colonies on potato-dextrose agar. It was immediately suspected that this organism was the cause of the disease. Inoculation experiments were carried out on Russian dandelion plants in the greenhouse with pure cultures of this bacterium, and lesions typical of those observed in the field were produced. Infection resulted when uninjured leaves were atomized with a water suspension of a young culture of the bacterium, and also occurred where the leaves were pricked with the point of a fine needle.

In the older leaves the lesions were extensive and spreading, while on young leaves they generally remained small and rarely exceeded 1 or 2 mm. in diameter. From these artificially induced lesions the same organism was consistently reisolated in pure culture.

In the field the roots have not been observed to be affected by the bacterium, and several attempts to induce infection of the roots through artificial inoculation were not successful.

Repeated inoculations on the common dandelion (*Taraxacum officinale* Weber), Cos lettuce (*Lactuca sativa* L. var. *longifolia* Lam.), head lettuce (*L. sativa* L. var. *capitata* L.), leaf lettuce (*L. sativa* L. var. *crispa* L.), and prickly lettuce (*L. scariola* L.) all failed to give infection.

Sigriansky (6) reported a "black necrosis" of the related Russian species, *Scorzonera tau-saghyz* Lipch. and Bosse, and gave the following description of the disease: "First to appear are small, black dots, which quickly coalesce to form much larger spots. The secretion of ooze drops has been

[1] Cost of publication borne by an outside agency.

[2] The writer wishes to express his appreciation of the indispensable help and advice given by Professor W. H. Burkholder during the progress of this work.

seen. The disease may be spread throughout the field, causing a premature dying of the leaves. The pathogen is apparently carried over in the seeds, and is spread with the help of insects, the wind, or soil particles, and especially in irrigating." This brief description suggests the same disease as that found at Ithaca on *Taraxacum kok-saghyz,* and also indicates that the pathogen may have been introduced with the seed imported from the Soviet Union. Sigriansky did not identify the pathogen except to infer indirectly that the causal agent is a bacterium.

In making the routine tests to describe the causal organism, 7 separate isolates were used, 1 of which descended from a single cell. All 7 isolates behaved similarly. In making the measurements the Congo red negative stain was employed. Plimmer and Paine's (5) modification of the Casares-Gil flagella stain was used to demonstrate the polar flagellum. The test for indol production in tryptophane broth was performed by the Ehrlich-Bohme technique (2). To determine the production of hydrogen sulphide from tryptophane broth, strips of filter paper impregnated with lead acetate were suspended over the cultures. The synthetic nitrate medium, described in the Manual of Methods (7), was used in the nitrate studies. Clara's dilute medium (3) was used to determine whether asparagin could be used as a combined carbon and nitrogen source. The lipase test was made according to the method outlined by Starr (8).

In making the biochemical tests to determine substances that can be used as a carbon and energy source, a synthetic basal medium, as given in the Manual of Methods (7), was prepared, with brom thymol blue added as an indicator. This basal medium was adjusted to pH 7, tubed, and autoclaved. The carbohydrates to be tested were prepared in 7.5 per cent aqueous solutions, sterilized by filtration, and then added aseptically to the tubes of basal medium, so that 0.75 per cent solutions were obtained. Sodium salts of the organic acids were added directly to the basal medium so as to make a 0.15 per cent solution, and autoclaved together. The test for the hydrolysis of starch was made by the starch-agar iodine method.

## DESCRIPTION OF PATHOGEN

*Morphology:* The bacterium is a rod and occurs singly or in pairs. Individuals from a 24-hour culture on potato-dextrose agar at 27° C. give measurements of 1.4–3.3 $\mu \times .7$–1.2 $\mu$ (average 0.9 $\mu \times 2.3\ \mu$). It is motile with one polar flagellum, and is Gram-negative. No spores are formed.

*Cultural Characters:* On beef-extract peptone agar the colonies are circular, smooth, and bright yellow; growth is moderate. On potato-dextrose agar growth is abundant, pale-yellow, mucoid, and wet-shining. Beef-extract peptone broth becomes turbid, usually with a thin ring. In milk, growth is good and litmus is reduced; a soft casein curd is precipitated and then slowly digested; the supernatant liquid gradually clears and tyrosine crystals are abundantly produced. The optimum temperature for growth is about 30° C. Maximum and minimum temperatures for growth are 38° C. and 0° – 3° C., respectively.

*Biochemical Characters:* Growth in gelatin is good, and liquefaction occurs rapidly. No gas is produced in a beef-extract peptone broth plus 1 per cent dextrose. Growth in tryptophane broth is good; hydrogen sulphide is produced but not indol. Nitrate and ammonium salts can be utilized as a source of nitrogen; asparagin cannot be used as a collective carbon and nitrogen source. Nitrates are not reduced to nitrites. Lipase is produced. Acid is produced from xylose, dextrose,

galactose, levulose, lactose, sucrose, and glycerol; no growth occurs with arabinose, maltose, raffinose, inulin, mannitol, ethanol, and salicin. Salts of acetic, citric, lactic, malic, and succinic acids are utilized with an increase in pH. Salts of formic, tartaric, salicylic, and benzoic acids are not utilized. Starch is hydrolyzed. Sodium chloride tolerance is between 3.25 and 3.5 per cent.

To the writer's knowledge there is no report in the literature of a species of bacterium pathogenic to the Russian dandelion, *Taraxacum kok-saghyz* Rod., and the species described here is distinct in pathogenicity, as well as in several morphological and biochemical characters, from *Phytomonas lactucae-scariolae* Thornberry and Anderson (9) and from *P. lactucae* (Yamamoto) Burkholder as given by Bergey *et al.* (1)[3]. The bacterium is hence described here as new. Its characters listed above designate it as a species of *Xanthomonas* Dowson (4); and the name proposed is *Xanthomonas taraxaci* n. sp.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY,
ITHACA, NEW YORK.

LITERATURE CITED

1. BERGEY, D. H. *Phytomonas lactucae* (Yamamoto) Burkholder. *In* Bergey's Manual for Determinative Bacteriology, p. 163. 1939.
2. BOHME, A. Die Anwendung der Ehrlichschen Indolreaktion für bacteriologische Zwecke. Zentralbl. f. Bakt. 1: 40: 129–133. 1905.
3. CLARA, F. M. A comparative study of the green-fluorescent bacterial plant pathogens. New York (Cornell) Agr. Exp. Stat. Mem. 159: 8. 1934.
4. DOWSON, W. J. On the systematic position and generic names of the Gram-negative bacterial plant pathogens. Zentralbl. f. Bakt. 2: 100: 177–193. 1939.
5. PLIMMER, H. G. and S. G. PAINE. A new method for the staining of bacterial flagella. Jour. Path. and Bact. 24: 286–288. 1921.
6. SIGRIANSKY, A. M. (Russian title). Rubber bearers. *In* Handbook for the agronomist on the control of diseases of agricultural plants, pp. 272–275, fig. 270–272. 1936.
7. Society of American Bacteriologists, Committee on Bacteriological Technique. Manual of methods for the pure culture study of bacteria. (Geneva, N. Y.) — 1923 to date. Leaflet II, ed. 8, 1942, p. 15.
8. STARR, M. P. Spirit blue agar; a medium for the detection of lipolytic microorganisms. Science 93: 333–334. 1941.
9. THORNBERRY, H. H. and H. W. ANDERSON. Comparative studies on cultures of *Phytomonas lactucae-scariolae* n. sp. and *Phytomonas pruni*. Phytopath. 27: 109–110. 1937.

[3] Original article is in Japanese.

## PHYTOPATHOLOGY NOTE

*Prevention of Three Peach Diseases by Ferric Dimethyldithiocarbamate Spray.*—In 1942 ferric dimethyldithiocarbamate (Fermate) was tested as a spray for control of 3 peach diseases occurring in Sacramento Valley, California, peach orchards.

Two treatments, 27 and 13 days, respectively, before harvest (September 8) were given for brown rot of ripening fruit, caused by *Sclerotinia fructicola* (Wint.) Rehm. On the day harvest started the incidence of fruit infection in one orchard was: nonsprayed, 11 per cent; Fermate 1 lb. to 100 gal. plus 4 oz. of a wetting agent to 100 gal., 4 per cent. In a second orchard the incidence of fruit infection was: nonsprayed, 19 per cent; Fermate 1–100 plus 4 oz. of a wetting agent to 100 gal., 4 per cent; liquid lime-sulphur 0.75–100 plus 4 oz. of a wetting agent to 100 gal., 8 per cent. All percentages are averages of 4 replications.

Peach trees were sprayed October 16 with Fermate, liquid lime-sulphur, and Bordeaux mixture. Within 1 week after spraying, rust caused by *Tranzschelia pruni-spinosae* developed abundantly on leaves of unsprayed trees. To determine the control by the sprays, counts were made October 28. According to the results in column 2, table 1, Fermate prevented leaf infec-

TABLE 1.—*Comparative effectiveness of different spray materials in the control of rust (Tranzschelia pruni-spinosae) and blight (Coryneum beijerinckii) on peach*

| Treatment | Average number of lesions of rust per leaf | Average number of lesions of blight per 100 twigs |
|---|---|---|
| Unsprayed | 18 | 156 |
| Fermate 1.5–100 | 3 | 5 |
| Fermate 1.5–100 plus lime 1–100 | 1 | 12 |
| Bordeaux mixture 10–10–100 | 9 | 23 |
| Lime-sulphur 4–100 | 1 | 65 |
| Lime-sulphur 6–100 | 1 | 14 |

tion during this period equally as well as lime-sulphur and considerably better than Bordeaux mixture. There was a suggestion that the addition of lime to the Fermate increased its effectiveness.

Within 1 month after application of the sprays in this orchard, blight or shot-hole, caused by *Coryneum beijerinckii,* developed to a moderate extent on twigs. The control obtained with the different sprays during this period is given in column 3 of the table. Fermate appeared to be significantly more effective against blight than lime-sulphur 4–100, and as effective as lime-sulphur 6–100 and Bordeaux mixture 10–10–100.

It should be noted, however, that in general practice of peach spraying one application of spray in the autumn is expected to protect the twigs against infection by rust and blight until the beginning of the next season. In such circumstances the spray deposit is subjected to weathering for a

period of several months, and, in consequence, weather resistance becomes an important factor in determining the effectiveness of the spray. In the present tests, which covered periods of 12 days in case of rust and 1 month in case of blight, weather resistance of the spray deposit was not so thoroughly tested.—E. E. WILSON and C. E. SCOTT, Division of Plant Pathology and Agricultural Extension Service, respectively, University of California, Berkeley, California.

*Bacterial Infection and Decay of the Inner Wood of Winter-injured Young London Plane Trees.*—The unusual occurrence of frost cracks in 5-year-old London plane trees (*Platanus acerifolia* Willd.) on bottom land in a Maryland nursery was called to the attention of the writer in 1934. Pruning wounds that were not healing properly were noticed the following summer on many of the trees. Trees thus affected presented a generally unhealthy appearance.

All apparently unhealthy trees were found on examination to have their 3 innermost annual rings discolored and water-soaked. This condition terminated abruptly about 5 inches aboveground. This water-soaked internal condition reached the surface of open pruning wounds; and a condition resembling slime flux was present. This apparently acted to prevent callusing. In addition, many of the open pruning wounds apparently had served as a point of entry to a rot fungus. This fungus had decayed the tissues of the inner 3 rings as much as 6 inches in each direction from the pruning wound. Often the rot fungus had entered at more than one point on the tree and the decay was almost continuous. A number of the trees died or broke off. Frost cracks occurred in half of the diseased trees. Every tree with frost cracks was found to be one with water-soaked inner wood.

From the decayed portion of the wood a hymenomycete was isolated. No fruiting bodies were found on any of the trees, but tissue cultures of the fungus were compared by R. W. Davidson of the Division of Forest Pathology with those in the wood-decay collection of that Division and found to be similar to known isolates of *Polyporus versicolor* L. ex Fr.

From the water-soaked, discolored inner wood, Gram-negative, $0.25 \times 0.5\ \mu$, rod-shape bacteria were consistently isolated. Preliminary inoculation tests indicated that these bacteria were capable of killing green shoots of newly rooted plane tree cuttings, when inoculated into a needle prick, or the entire cutting, if inoculated through a cut in the bark. What appeared to be similar bacteria were isolated from cuttings that failed to callus well. That the bacteria did not readily attack healthy tissue of well-established trees was indicated when examination of some of the survivors of the original group of trees several years later showed the water-soaked condition still limited almost entirely to the innermost 3 rings.

The most probable explanation of the observed phenomena is in the conditions of the preceding winters. The winter of 1931–2 was unpre-

cedentedly warm from November to February, inclusive. Many kinds of plants started growth prematurely. This was followed by cold weather in March. From the 10th to the 13th, inclusive, minima were between 15° and 18° F. at the College Park Weather Station, located on higher and presumably warmer ground, and on the 19th 11° F. was recorded. A considerable snowfall also occurred. Killing or injury of parenchyma in the wood is supposed to have resulted, followed by invasion of the bacterium and the decay fungus. The general limitation of the infections to wood above the snow line and to the rings formed before the 1932 freeze supports this view. Wetwood and slime flux are commonly associated with bacterial infection of the wood. The frost cracks, probably limited to the wetwood trees, occurred during the subzero weather of February, 1934, and are regarded as consequences of the water-soaked condition.—BOWEN S. CRANDALL, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Athens, Ga.

The votes regarding formation of a Potomac Division have been canvassed. The following are the results:

226—voting in favor of the formation of a Potomac Division

81—opposed to the formation of a Potomac Division

This vote indicates approval of the Society for the formation of a Potomac Division as outlined in the petition presented to the membership.

Special Committee:

Signed: W. G. STOVER, *Chairman*
C. W. ELLETT
D. M. MCLEAN

# DECAY IN MERCHANTABLE BLACK CHERRY ON THE ALLEGHENY NATIONAL FOREST[1]

ROSS W. DAVIDSON[2] AND W. A. CAMPBELL[3]

(Accepted for publication May 7, 1943)

## INTRODUCTION

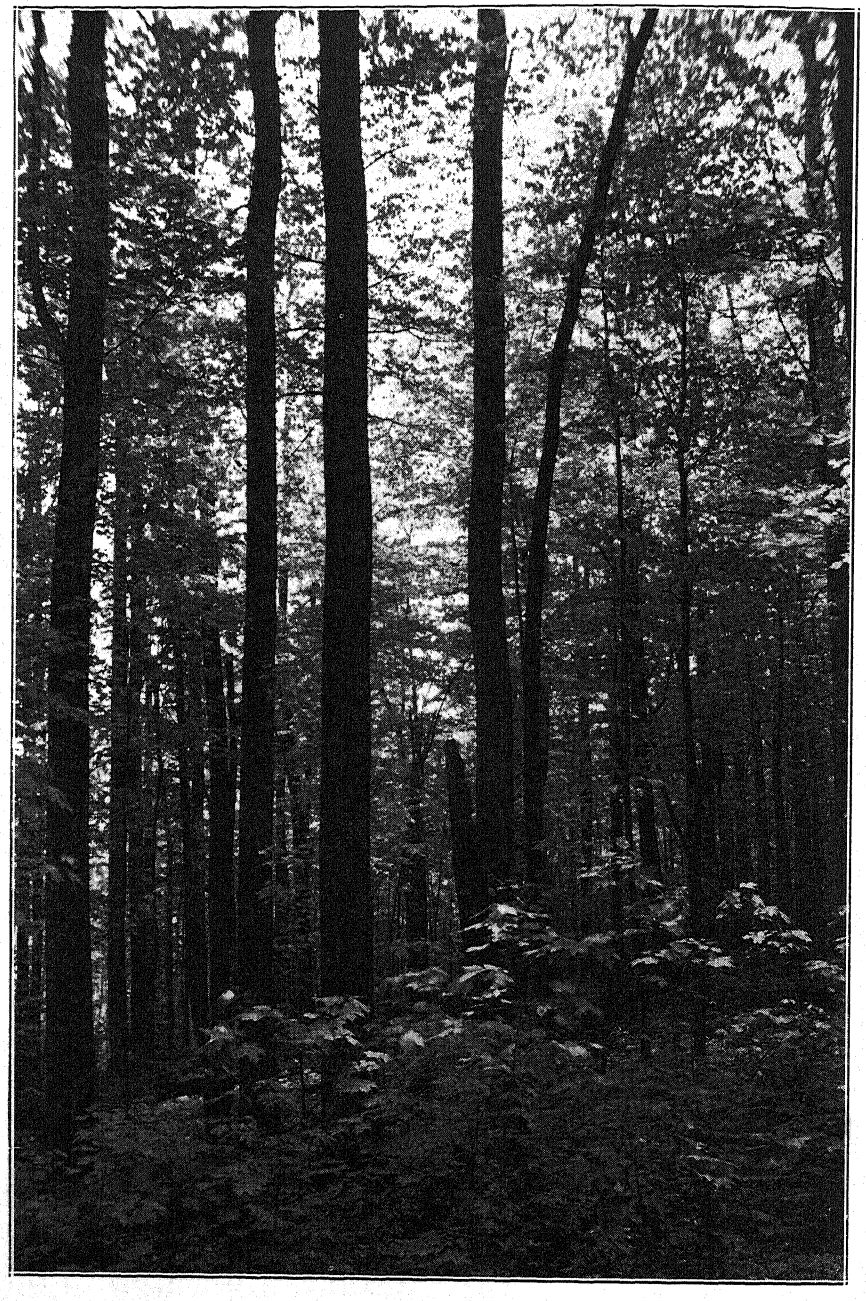
The botanical range of the black cherry (*Prunus serotina* Ehrhart) includes most of the eastern half of the United States and adjacent areas of eastern Canada. It is commercially important as a timber tree from West Virginia to western New York. Black cherry is one of the most important tree species in the unburned, second-growth, northern hardwood stands of the Allegheny Plateau in Pennsylvania, and, according to Ostrom (15), becomes more important as the number of short cutting cycles increases. Downs (11) has shown that in the mixed hardwood stands of northwestern Pennsylvania black cherry is abundant in the second growth, and both seedlings and sprouts gain early dominance over the slower growing tolerant species. He states that such stands generally become two-storied, the black cherry forming a large-crowned overstory, while the suppressed and intermediate sugar maple, yellow birch, and beech occupy subordinate positions and are of smaller size for the same age. Based on cubic-foot volume these second-growth stands were composed of black cherry 34.2 per cent, sugar maple 19.8, beech 14.6, yellow birch 8.1, and red maple 6.9 per cent. The 1936 glaze storm reduced black cherry to 20.6 per cent of the total volume, second to sugar maple, which was raised to 26.5 per cent. Black cherry probably will not produce lumber of such good quality as the slower growing sugar maple because its dominant position in the stand results in more limby trunks and greater damage from glaze.

Black cherry was early considered valuable for furniture and engraver's blocks; and most of the old-growth stands have been cut. Black cherry lumber now comes largely from second-growth stands and good quality material is difficult to obtain. Its suitability as pulp wood has increased its demand by paper mills operating in the Allegheny Region. This species also is used for chemical wood, but is not considered so desirable as are beech and maple. When this study was made, stumpage value of black cherry was intermediate between that of beech and sugar maple. With the rapid increase in volume in the second-growth stands now coming into timber production, its value should increase.

[1] In cooperation with the Allegheny Forest Experiment Station and the Allegheny National Forest. Special thanks are due A. F. Hough, J. C. McClellan, and J. B. Smith for their assistance.

[2] Associate Mycologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[3] Formerly Assistant Forest Pathologist, Division of Forest Pathology; now Associate Forest Pathologist, Special Guayule Research Project, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.



*(Photo by A. F. Hough.)*

FIG. 1. Nearly pure 116-year-old black cherry on Camp Run, near Westline, Pennsylvania. These trees were from 10 to 26 inches d.b.h. and had an average merchantable length of 51 feet. Many of the trees had small crowns, which were bady damaged by the 1936 glaze storm. Very little reproduction had established itself under the denser portions of the stand, but the larger openings had seeded in almost solidly to sugar maple.

The present paper gives the results of a study of decay in merchantable black cherry. This information should be of value in managing these second-growth stands for high quality timber production.

## SOURCES OF MATERIALS AND METHODS

### Areas Studied[4]

The investigators had available for the intensive part of this study a 20-acre sale area of dense, almost pure old-growth black cherry, 116 years old at Camp Run, near Westline, Pennsylvania (Fig. 1). The trees there were tall and straight and ranged from 10 to 26 inches d.b.h. and 100 to 115 feet in total height, with an average merchantable length of 51 feet (Table 1).

TABLE 1.—*Comparative information on merchantable black cherry trees in three logging areas on the Allegheny National Forest*

| Area | Basis | Average age | Average stump diameter | Average merchantable length[a] | Total volume | Cull | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Butt rot | Trunk rot | Total rot | Per cent of rot |
| | *Trees* | *Years* | *Inches* | *Feet* | *Bd.ft.* | *Bd.ft.* | *Bd.ft.* | *Bd.ft.* | *Bd.ft.* |
| Four Corners | 21 | 52 | 18.5 | 33 | 3,980 | 90 | 0 | 90 | 2.3 |
| Libby Run ... | 20 | 120 | 25.7 | 46 | 13,520 | 75 | 750 | 825 | 6.1 |
| Camp Run ..... | 281 | 116 | 20.1 | 51 | 101,910 | 2,615 | 8,935 | 11,550 | 11.3 |

[a] Includes only logs cut and milled and logs that would have been milled had they not been culled for decay. Portions of trunks left in the woods because of bad checks or bad form were not included in merchantable length or board-foot volume.

On Libby Run, also near Westline, Pa., a small sample was taken from a 120-year-old stand of more open-grown cherry. The trees on this area were mixed with other hardwoods and were larger in diameter than those at Camp Run. They had large branching tops and were from 16 to 28 inches in diameter, with an average merchantable length of 46 feet.

At Four Corners, 12 miles southeast of Kane, Pennsylvania, a third sale area, which consisted of scattered old-growth maple and beech interspersed with groups of 52-year-old black cherry and other hardwoods also was sampled. The merchantable cherry, which was open-grown with large branching tops, was from 11 to 20 inches in diameter with an average merchantable length of 33 feet.

[4] Mr. A. F. Hough has kindly supplied additional data on these stands. He states that the Libby Run cherry originated from a blowdown of virgin beech-maple forest 120 years prior to 1940 and Camp Run after a blowdown of virgin beech-maple forest 116 years prior to 1940. The Four Corners cherry originated after a heavy cutting of hemlock-beech forest and the trees became limby and forked due to lack of crowding by trees of similar growth rate.

The Libby Run area had been subjected to logging in 1894. From the Camp Run stand 4 M.B.F. in sawlogs of various sizes was selectively cut during the period 1914 to 1940. As computed on sample plots 21 M.B.F. per acre was left, which grew at the annual rate of 485 board feet per acre. At the time of the 1940 cutting, a total volume of 34 M.B.F. per acre had been produced by the residual trees, disregarding all natural mortality.

In addition to the study of decay in these 3 merchantable stands a general search of many areas in the Allegheny National Forest was made for cherry heartwood-decaying fungi, particularly for those producing conks on living or dead trees. Whenever possible, cultures were obtained from these conks for comparison with cultures from heartwood decay.

### Method of Collecting Data

Information on individual trees was obtained by following the logging crews on the areas sampled. Stump and top diameters and length of each log were recorded for all sound and decayed trees. The diameter of decay on the ends of the logs was recorded and the extent of decay was measured or estimated. Isolations of the causal fungi were made from decay samples, and these were identified by comparison with isolates from identified conks.[5]

### Method of Scaling

The Scribner Decimal C log rule, standard with the Forest Service on the Allegheny National Forest, was used in scaling all logs. In determining volume, only the merchantable portion of the trunk or what would have been, except for decay, was included as total merchantable volume. Parts of trunks left in the woods because of butt check or bad form were not included. No attempt, however, was made to subtract cull for check, sweep, and defects other than rot from logs taken to the mill.

## DECAY IN RELATION TO TYPE OF STAND

Some stands contain much decay, whereas others contain very little; a difference often difficult to explain. Usually, however, decay reflects age, severe fire damage, or other factors. The difference in the amount of cull from decay on the 3 areas studied (Table 1) can be explained in part by the difference in age. The black cherry at Four Corners was only 52 years old, or less than half the age of the stands at Libby Run and Camp Run. As would be expected, cull from decay was only 2.3 per cent, as compared to 6.1 and 11.3 per cent, respectively, in the two older stands. Also, the decay that caused cull in the Four Corners trees was mostly that of the butts, whereas in the others it was chiefly in trunks or tops.

In comparison with the Camp Run and Libby Run stands, the Four Corners' black cherry contained more butt rot than was expected in such young trees. The data (Table 1) do not account for this difference, and no reason for it was found in a more detailed study of the area. The 21-tree sample possibly did not suffice for accurate information, but a thorough survey of all cherry stumps over much of the sale area indicated that butt infections were as frequent as or probably more so than in the Camp Run stand. Theoretically, this may be explained by assuming that trees with butt decay did not survive for 116 years. Actually, however, very few 116-year-old trees were weakened sufficiently by butt rot to wind throw.

[5] The identifications of the fungi found fruiting on black cherry were made or verified by L. O. Overholts of Pennsylvania State College.

TABLE 2.—*Summary of cull caused by different fungi in 281 trees on the Camp Run Sale Area*

| Fungi | Distribution of cull | | Infections upon which cull is based |
|---|---|---|---|
| | *Per cent* | *Board feet* | *Number* |
| *Poria prunicola* | 36.6 | 4,225 | 50 |
| *Poria mutans* | 13.4 | 1,545 | 14 |
| *Fomes pinicola* | 9.0 | 1,040 | 21 |
| *Polyporus sulphureus* | 6.6 | 760 | 18 |
| *Polyporus spraguei* | 2.7 | 310 | 10 |
| *Trametes serialis* | 1.5 | 175 | 3 |
| *Poria sericeo-mollis* | 1.3 | 150 | 2 |
| *Polyporus berkeleyi* | 0.9 | 100 | 5 |
| *Poria inflata* | 0.8 | 95 | 5 |
| *Polyporus schweinitzii* | 0.6 | 70 | 2 |
| Miscellaneous fungi | 3.1 | 360 | 3 |
| Undetermined fungi | | | |
| Brown rot | 3.4 | 390 | 12 |
| White rots | 18.2 | 2,100 | 46 |
| Mixed rots | 2.0 | 230 | 8 |
| Totals | ..... | 11,550 | 199 |

Of the two older stands, the one at Camp Run was studied more carefully and yielded most of the information on types of decay, species of rot-inducing fungi (Table 2), and their extent and mode of entrance. From the standpoint of comparison with the Four Corners area, the Libby Run sample is more valuable, because these trees were open-grown and could be pictured as the Four Corners trees 58 years later. In spite of numerous overgrown branch stubs in the Four Corners trees, there were few infections by top-inhabiting fungi. A few *Fomes pinicola* (Sw.) Cke. conks were found by searching the entire area (Table 3) but no infections of *Poria prunicola* (Murr.) Sacc. and Trott., the principal cull-producing fungus in older stands, were found.

The Camp Run trees formed crowded stands. Most of them were growing very slowly and bore numerous dead branch stubs, a condition that had existed for years and had resulted in early infection by top-rotting fungi and a consequent high proportion of cull. The Libby Run trees bore large tops with numerous big dead branches and branch stubs fairly low down on the trunks. These contained numerous top infections, but only a small number resulted in cull.

## THE DECAYS OF BLACK CHERRY

### Points of Entrance

Decays may be divided into two groups depending upon their location in the tree. Those in the base are referred to as butt rots; those in the trunks as trunk rots. The stands studied on the Allegheny Plateau had not been injured by fires, and butt rots were mainly associated with logging wounds, dead roots, drouth injuries, or obscure basal lesions. Most of the

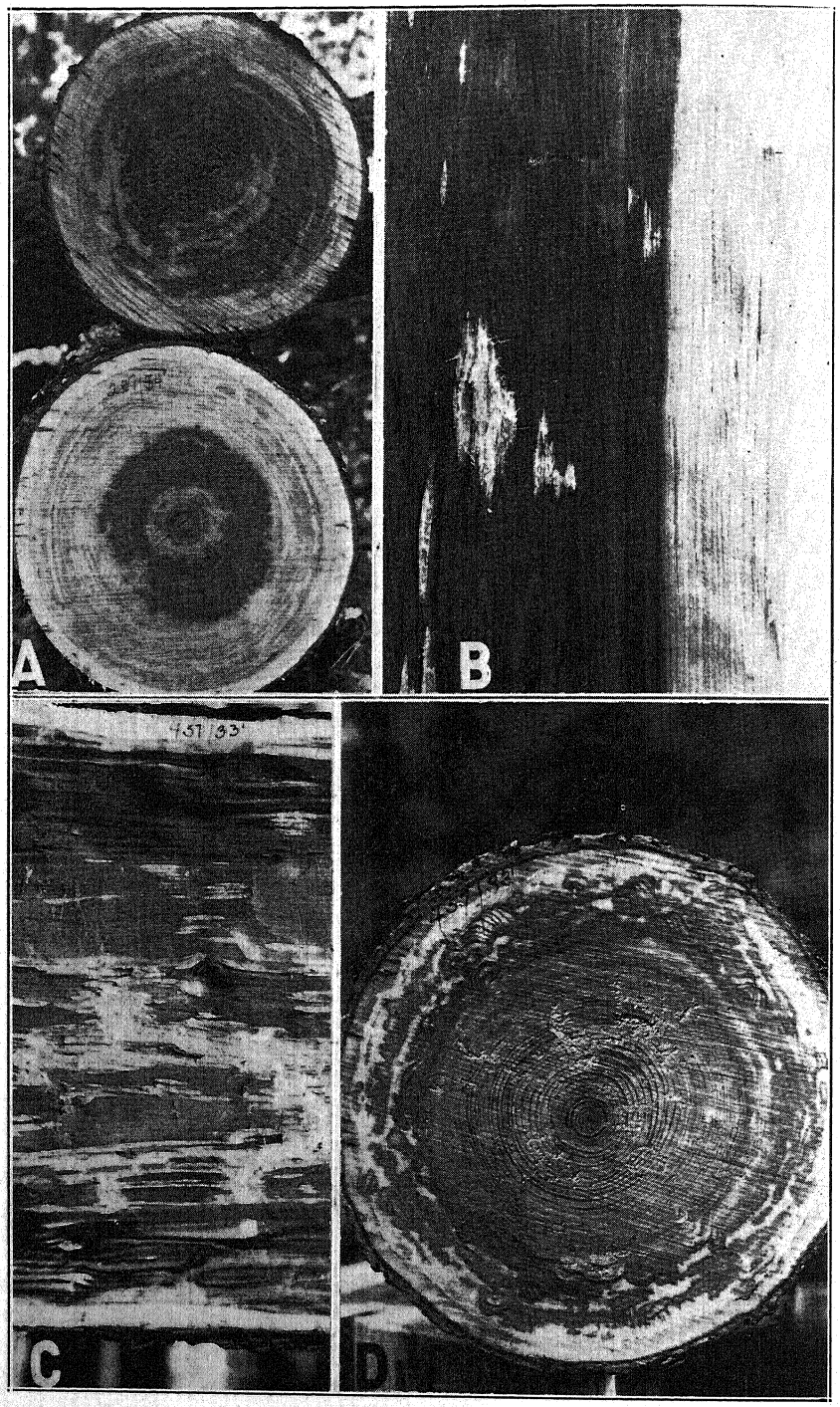



FIG. 2. *Poria prunicola* rot of black cherry. A. Red discoloration of the heartwood as seen on the end surfaces of recently cut logs. The cut end of the lower log was at 54 feet, the top log, corresponding to the opposite or top end of the same log, was at 66

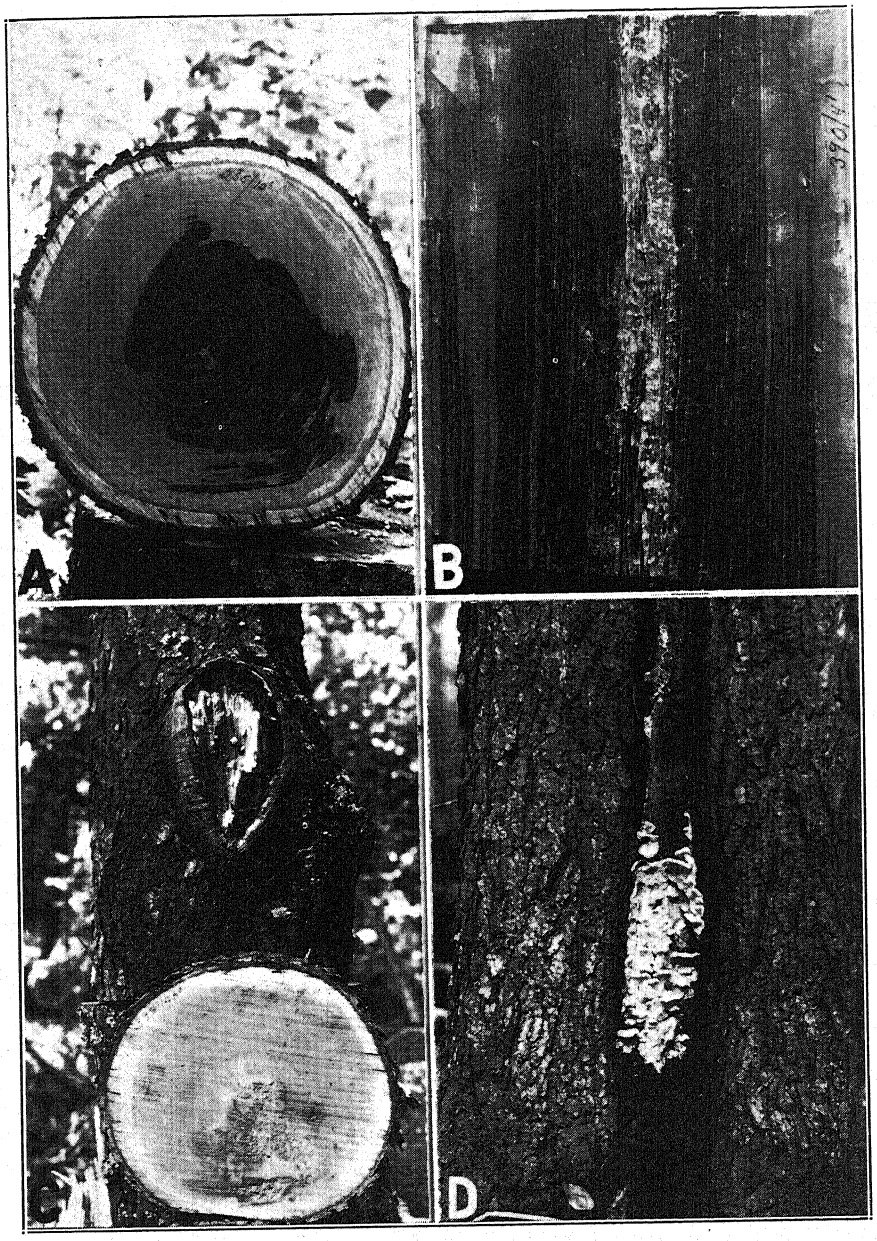



FIG. 3. A. and B. *Poria mutans* rot in black cherry. A. End cut showing the water-soaked nature of the rot. B. The pocket or piped advanced stage of the rot. C. A rot-infected branch stub approximately 4 inches in diameter. The brown rot, which had extended more than a foot below the stub, was caused by *Poria inflata*. D. Conks of *Polyporus subcartilagineus* on a butt wound. This fungus causes a brown rot.

feet. The rot infection originated above 66 feet in the tree and was working down the trunk. B. White pockets of advanced decay in the red-stained heartwood. C. A thoroughly rotted log split open to show the white pockets and streaks of advanced decay. These white pockets are not conspicuous on end cuts, even in badly rotted logs. D. Advanced decay in a thoroughly rotted top showing how the decay may encroach upon the sapwood.

TABLE 3.—*Summary of all fungi isolated from butt and trunk rot in black cherry*

| Fungi | Decay infections from all stands | | | Decay infections from Four Corners and other young stands | | | Decay infections from Camp Run and Libby Run | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total | Butt | Trunk | Total | Butt | Trunk | Total | Butt | Trunk |
| | *Number* | *Number* | *Number* | *Number* | *Number* | *Number* | *Number* | *Number* | *Number* |
| *Coniophora cerebella* Pers. | 8 | 8 | 0 | 7 | 7 | 0 | 1 | 1 | 0 |
| *Corticium lividum* Pers. ex Fr. | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| *Fomes pinicola* (Sw.) Cke. | 32 | 2 | 30 | 6 | 1 | 5 | 26 | 1 | 25 |
| *Hydnum* sp. | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 2 |
| *Omphalia campanella* Fr. | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 2 |
| *Polyporus balsameus* Pk. | 2 | 0 | 2 | 2 | 0 | 2 | 0 | 0 | 0 |
| " *berkeleyi* Fr. | 9 | 9 | 0 | 3 | 3 | 0 | 6 | 6 | 0 |
| " *fibrillosus* Karst. | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| " *frondosus* Dicks. ex Fr. | 2 | 2 | 0 | 1 | 1 | 0 | 1 | 1 | 0 |
| " *schweinitzii* Fr. | 2 | 2 | 0 | 0 | 0 | 0 | 2 | 2 | 0 |
| " *spraguei* Berk. and Curt. | 14 | 14 | 0 | 0 | 0 | 0 | 14 | 14 | 0 |
| " *subcartilagineus* Overh. | 3 | 2 | 1 | 2 | 2 | 0 | 1 | 0 | 1 |
| " *sulphureus* Bull. ex Fr. | 28 | 5 | 23 | 1 | 0 | 1 | 27 | 5 | 22 |
| " *versicolor* L. ex Fr. | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| *Poria cocos* (Schw.) Wolf | 3 | 3 | 0 | 2 | 2 | 0 | 1 | 1 | 0 |
| " *inflata* Overh. | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 |
| " *mutans* Pk. | 18 | 7 | 11 | 2 | 2 | 0 | 16 | 5 | 11 |
| " *prunicola* (Murr.) Sacc. and Trott. | 62 | 0 | 62 | 0 | 0 | 0 | 62 | 0 | 62 |
| " *sericeo-mollis* (Rom.) Baxter | 10 | 6 | 4 | 6 | 4 | 2 | 4 | 2 | 2 |
| " *xantha* (Fr. ex Lind) Cke. | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| *Stereum rameale* Schw. ex Burt | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| *Trametes serialis* Fr. | 5 | 3 | 2 | 2 | 0 | 2 | 3 | 3 | 0 |
| Totals | 212 | 66 | 146 | 35 | 22 | 13 | 177 | 44 | 133 |

trunk rots were either associated with elongated trunk wounds or with dead-branch stubs larger than 3 inches in diameter.

## Types of Rot

On the basis of color and texture, wood decays are classified as brown, carbonizing rots, or white, noncarbonizing rots. About two-thirds of the cherry rots are of the former type, and include those caused by *Fomes pinicola, Polyporus spraguei, P. schweinitzii, P. sulphureus, Poria cocos, P. sericeo-mollis* and *Trametes serialis.* The brown rots are very similar to each other in texture and color, and the fungi causing them usually can be identified only in pure culture.

The important noncarbonizing rots were caused by *Poria prunicola, P. mutans* and *Polyporus berkeleyi,* and can be identified usually on the basis of the following characteristics:

*White-flecked Red Rot* (*Poria prunicola*). Characterized by red discoloration of heartwood, extending sometimes throughout 20 to 40 feet of trunk. Ends of infected logs purplish-red covering most of end section or confined to smaller areas near center (Fig. 2, A). The red-colored wood is altered mechanically in definite pockets or areas of soft, white, flaky decay (Fig. 2, B). These pockets, few or abundant, are not conspicuous on end cuts, but plainly visible when an infected log is split (Fig. 2, B and C). Decay confined to heartwood except in old infections where it may encroach on sapwood (Fig. 2, D).

*White-streaked or Piped Rot* (*Poria mutans*). White, water-soaked, pocket or piped rot of heartwood (Fig. 3, A and B); may extend from injuries, up and down the trunk. The incipient stage generally dark and water-soaked (Fig. 3, A).

*Light-brown Flaky Rot* (*Polyporus berkeleyi*). A light-brown or yellowish, soft, flaky, butt rot (Fig. 4, A), recognized readily by peculiar sweet odor. Fungus probably enters small dead roots; conks were found several times on soil near bases of infected trees (Fig. 4, B).

### CONKS ON LIVING BLACK CHERRY

Conks of *Fomes pinicola,* more prevalent on living black cherry than those of other fungi (Fig. 5, A to C). Commonly found on the larger trees associated with branch stubs (Fig. 5, A and B), branch wounds, or trunk wounds exposing heartwood. They also may develop at base of tree from butt wounds or wounds left by death or severance of companion stems. Sporophores of other *Fomes* common to hardwoods were infrequent on black cherry.

Conks of *Poria prunicola,* common on dead fire cherry (*Prunus pennsylvanica* L. f.), throughout Allegheny National Forest. No conks found on living black cherry and the only one collected on that species was on a rotted log, approximately 12 inches in diameter, that had been in contact with the ground for some years.

Several species of rot fungi fruited infrequently over a period of years. In certain seasons *Polyporus sulphureus* was common on living trees and decaying logs and stumps. *Polyporus spraguei* fruited abundantly on

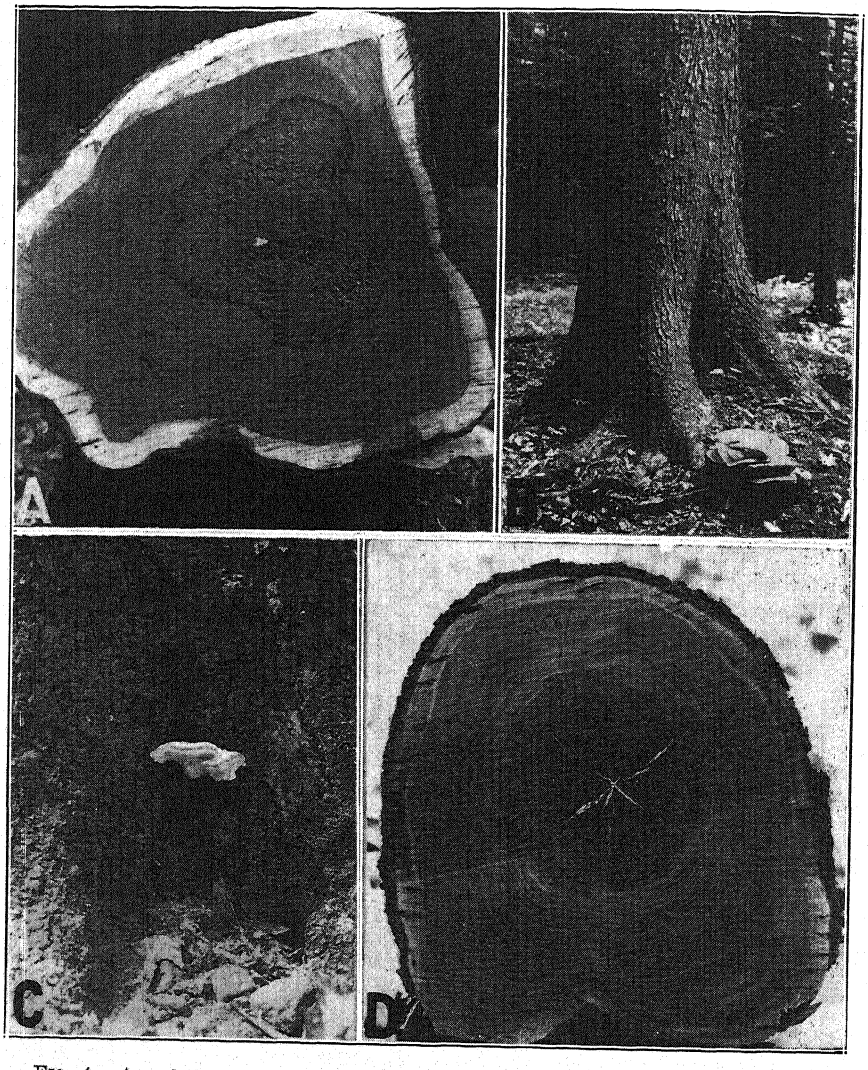



FIG. 4. A and B. *Polyporus berkeleyi* on black cherry. A. Butt rot as exposed by the stump cut. This rot was limited to the stump or extended but a short distance into the butt. B. A conk at the base of a large tree near Camp Run, photographed in August 1940. A conk was produced on the same spot in 1939. C and D. *Polyporus spraguei* on black cherry. C. A conk that developed from a small stump wound on a recently cut stump. This fungus did not fruit on living infected trees but was common at the base of dead snags or on the stumps of recently cut trees. D. Brown rot caused by the fungus.

recently cut stumps and cut surfaces of logs that exposed rot, but was not observed on living trees (Fig. 4, C). *Poria sericeo-mollis* fruited rather commonly on large wounds in living black cherry in 1936 and 1937. In

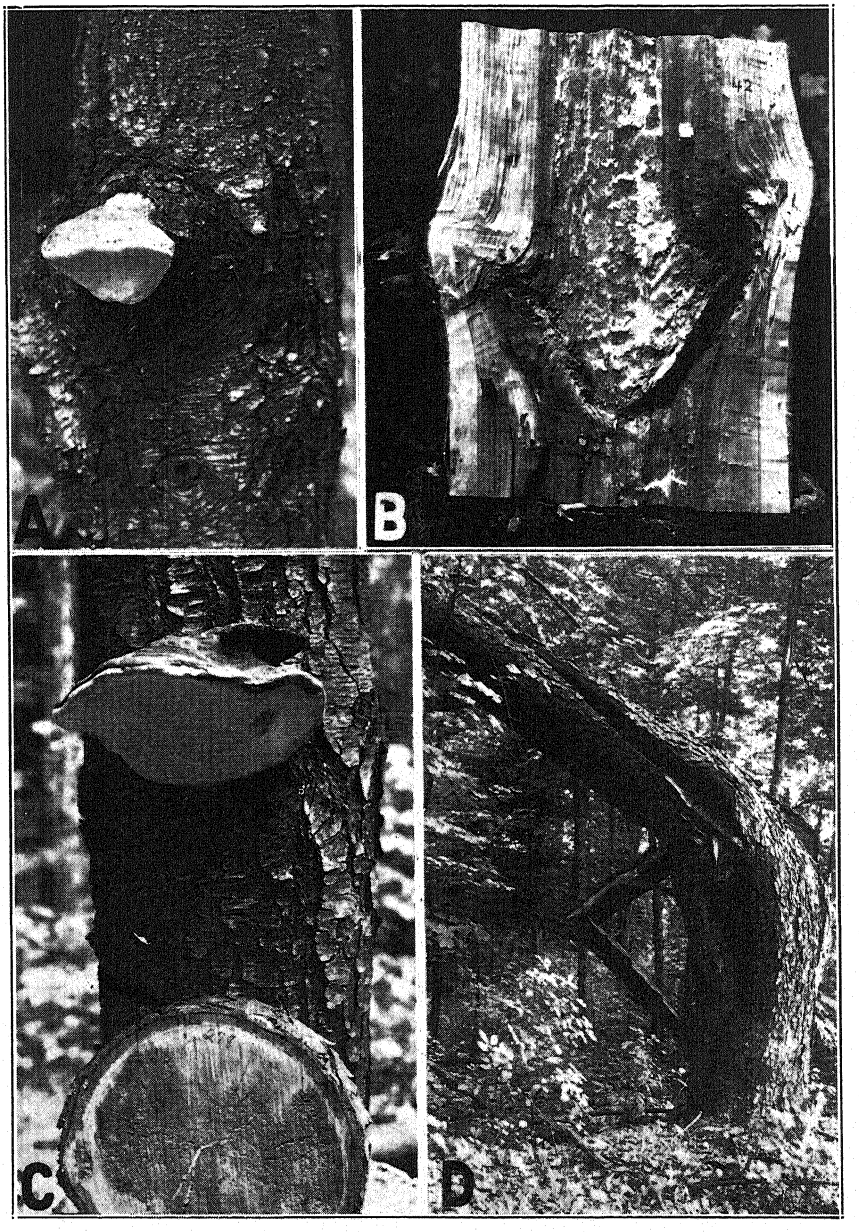



FIG. 5. A. to C. *Fomes pinicola* conks and rot of black cherry. A. Conk on a nearly healed 3-inch branch stub. B. An infected top split open to show the branch stub that served as the infection court and the badly rotted heartwood. C. A large conk associated with a branch stub on a 52-year-old tree. The diameter of the rot column one foot below the conk included practically the entire cross-sectional area of the heartwood. Badly rotted tops commonly break under strain caused by wind or glaze. D. A badly rotted black cherry approximately 24 inches d.b.h., which broke off in a windstorm.

1940, conks were not collected on living trees, but several were found on badly rotted logs. *Poria mutans* was observed fruiting abundantly on large trunk injuries in several instances. Most rot infections, however, were unaccompanied by fruiting. *Coniophora cerebella* was collected only on dead wood during a rainy period in the fall of 1940. A large fruiting body of *Polyporus berkeleyi* was collected from the base of a black cherry at Camp Run in 1939. A conk developed at the same spot again in 1940 (Fig. 4, B). One other fruiting body was found in the same area in 1940. *Polyporus schweinitzii* fruited in 1939 on one of the trees found to be infected with it at Camp Run. *Polyporus subcartilagineus* (Fig. 3, D) was found twice fruiting on decayed butt wounds, but the fungus was unimportant on the logging areas studied. *Polyporus balsameus* was collected once on the badly decayed surface of a large butt wound. *Trametes serialis* was collected once on rotten wood associated with a split fork.

The other heart-rotting fungi listed in table 3 were not observed to fruit on living cherry and were identified from cultures isolated from decay.

## PREDICTING CULL FROM DEFECTS IN STANDING TREES

Very few trees reach merchantable size without injuries, which serve as infection courts for wood-rotting fungi. Small injuries usually heal without decay, but large trunk and butt wounds, and particularly wounds caused by large branch stubs, heal slowly and often become badly rot-infected. Trees reserved for a future cut, if they already possess large wounds or are injured in logging, often decrease rather than increase in merchantable volume after a period of years, because of advancing decay. A number of black cherry trees on the different areas had conspicuous trunk and butt wounds. In table 4 these are analyzed as to size in relation to age of stand and extent of rot resulting in cull. These data include all trees with larger trunk and butt wounds, since these were easily recognized.

Broken branches and dead stubs were very common in stands of all ages. Many of these breaks were of recent origin and dated from the glaze storm of 1936. Older breaks were especially numerous in the 116-year-old trees, and were associated with approximately half of the decay cull found in the stands. In many trees decayed by *Poria prunicola,* the branch stubs that served as entrance courts had healed over completely. Most of the stems could not be dissected to determine the size of the stub responsible for infection, but, for the small number examined critically, the stub diameter was found to be over 3 inches. Thirty-two dead infected stubs in the 116-year-old trees had an average diameter of 6.5 inches with an average rot extent of 15 feet. All of these stubs were "old," that is they were rotten or were cavities in the trunk from which the stub itself had disappeared. Only 2 rot-infected stubs under 3 inches in diameter were noticed and these did not result in cull. Decay from branch stubs in black cherry rarely causes serious cull for 10 to 15 years. No cull was caused by *Polyporus*

*versicolor* and *Stereum rameale*, 2 fungi that were found to cause limited rot infections in top breaks caused by glaze (4).

TABLE 4.—*Extent of rot and cull associated with butt and trunk wounds in black cherry*[a]

| Stand age | Butt wounds | | | | Trunk wounds | | | | Split forks[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number | Average length wound | Average extent rot | Average cull | Number | Average length wound | Average extent rot | Average cull | Number | Average extent rot | Average cull |
| *Years* | | *Feet* | *Feet* | *Bd.ft.* | | *Feet* | *Feet* | *Bd.ft.* | | *Feet* | *Bd.ft.* |
| 116 | 10 | 5.5 | 10.2 | 100 | 13 | 10.6 | 17.3 | 150 | 2 | 18.5 | 90 |
| 52 | 11 | 4.3 | 6.5 | 30 | 1 | 3.0 | 8.0 | 60 | 5 | 11.4 | 60 |

[a] Includes all open wounds and readily detected healed wounds.
[b] If a fork had broken completely off the wound was classified as a trunk wound.

Only one fungus, *Fomes pinicola,* formed conks regularly on black cherry, and this fungus was found fruiting on 16, or half, of the trees it had infected. Most of these conks were in the tops in connection with dead stubs. Twelve conks of *Fomes pinicola* in the 116-year-old trees were associated with an average rot extent of 13.5 feet, and 4 conks in the 52-year-old trees were associated with 10.8 feet of rot. Conks of *F. pinicola* on a dead stub did not indicate more decay than the average decay associated with 32 stubs analyzed for the 116-year-old trees.

Some suggestions for estimating cull in black cherry are given in table 5.

## DECAYS IN BLACK CHERRY IN RELATION TO MANAGEMENT

One reason for studying decay in a particular tree species is to determine if cull from rot will affect management for saw-timber production. Another reason is to determine how intensive management may affect the incidence and amount of decay.

The present study indicates that decay will not be serious during the length of time necessary to produce a maximum yield of high quality black cherry saw timber. The amount of decay in the 116-year-old trees at Camp Run, although causing 11.3 per cent cull, was not excessive; and 116 years seems adequate for a satisfactory yield of good quality logs.

Several of the 120-year-old trees yielded approximately 1,000 board feet each; several on the Four Corners area yielded 300 board feet in 52 years. These faster-growing trees did not contain as high quality clear lumber as the slower growing ones, but many trees at Camp Run contained a fair amount of fine clear lumber. If the Camp Run stand had been thinned carefully during the last 60-year period the final stand would probably have contained the same volume in fewer trees and may have had less decay. This conclusion is based on the fact that the Libby Run trees, which grew under less crowded conditions, contained a smaller proportion of decay.

However, trunk injuries resulting from selective logging would probably increase the amount of decay.

Some might question the ability of black cherry to withstand crowded conditions necessary for good height growth during the early development of the stand, but the Camp Run stand contained many trees of small diameter which had survived years of crowding. Also much small-diameter 52-year-old cherry at Four Corners had attained an excellent height of clear stems. These trees will probably produce a greater volume of fine-quality lumber during the succeeding 50-year period than was obtained from the

TABLE 5.—*Summary of suggestions for estimating cull defect in merchantable black cherry from wounds or dead stubs 10 years old or older*[a]

| Type of injury | Age of stand | Extent of rot in relation to wound size |
|---|---|---|
| Butt or trunk wounds | 40 to 70 years | Average extent of rot 1.5 times length of visible injury<br>Average diameter of rot column equal to width of wound at widest part |
| | 70 to 116 years | Average extent of rot 2.0 times length of visible injury<br>Average diameter of rot column twice width of wound at widest part |
| Branch stubs | 40 to 70 years | Ordinarily rot from stubs not sufficient to cause appreciable cull |
| | 70 to 116 years | Average rot extent 15 feet below rotten knot holes or badly decayed branch stubs over 3 inches in diameter. Greatest danger from rot comes from stubs over 5 inches in diameter |
| Conks of *Fomes pinicola* | All ages | Average rot extent approximately 12 feet; above and below conk. Usually the larger the conk the more rot |

[a] Based on the observation that in general the older the trees, the longer they have been exposed to infection, and the older the wounds tend to be. Usually it takes from 10 to 20 years for black cherry wounds to become badly rotted.

faster-growing 52-year-old ones. This will depend upon how well cherry of this type responds to opening up of the stand, and how much damage will result from wind and glaze storms.

Butt rot was not important from the standpoint of cull in merchantable trees, even though a number of strictly butt-rot fungi were present. In only a few trees had butt rot developed an excessive amount of cull or had become sufficiently extensive to cause serious weakening (Fig. 5, D).

Stand-improvement operations obviously should attempt to remove forked trees and eliminate multiple sprout clumps or reduce them to single stems at an early age. Other outstanding evidences of poor-risk trees are large trunk wounds and sporophores of *Fomes pinicola*. Dead branch stubs do not indicate individual poor-risk trees, but a stand with many large dead branches will eventually have numerous decay infections.

From the standpoint of decay, black cherry should be an excellent tree to manage for lumber production. It has a durable heartwood and is

affected by relatively few strictly heartwood-inhabiting fungi. Black cherry also grows rapidly and most heartwood decayers do not have time to cause excessive cull before merchantable size is attained.

### DESCRIPTION OF BLACK CHERRY DECAY FUNGI IN CULTURE

The most practical means of determining the fungus responsible for decay in a particular piece of infected wood is by pure-culture methods. These methods have been described in detail by Davidson, Campbell, and Vaughn (10). By means of the pure-culture method 22 fungi were found to cause decay in black cherry on the Allegheny Plateau (Table 3). Nine of these, namely, *Corticium lividum, Polyporus berkeleyi, P. frondosus, P. spraguei, P. sulphureus, P. versicolor, Poria cocos, P. inflata,* and *Stereum rameale,* occurred as heart rotters in oak, and their cultural characteristics have already been described in detail (10). These fungi are listed in the key but no further description will be given. *Polyporus schweinitzii,* a species common to softwoods, has been described culturally by Childs (8) and further description of its cultural characteristics does not seem necessary. Only a few isolates were obtained for *Omphalia campanella, Polyporus fibrillosus,* and *Hydnum* sp. and these were not considered adequate for a description of the species in culture.

Detailed descriptions of cultures are given for 9 fungi that caused more or less extensive decay and for which a sufficient number of isolates from black cherry or other hosts were available to give some idea of the range of variations to be expected of each fungus in culture. These fungi are: *Coniophora cerebella, Fomes pinicola, Polyporus balsameus, P. subcartilagineus, Poria mutans, P. prunicola, P. sericeo-mollis, P. xantha,* and *Trametes serialis.*

#### Key to Black Cherry Decay Fungi, When Grown on Malt Agar

The following key is based on a classification system developed for oak fungi (10) and includes 9 fungi common to both oak and black cherry and 9 isolated only from black cherry. The cultural characteristics of the fungi causing rot in oak can be directly compared with those from black cherry by combining the two keys.

The first letter refers to mat color in 14 days in diffused light at room temperature for mats grown on malt agar in Petri dishes. A, white at all ages; B, at first white becoming yellowish or brownish in 14 days; C, yellow; D, brown; E, pink or orange; F, any other color not fitting in well with the first 5 divisions.

The second letter refers to the oxidase reaction, which is obtained by growing the fungi to be tested on malt agar to which has been added 0.5 per cent gallic or tannic acid (9). Brown-rot fungi usually cause no reaction or discoloration of these media; white-rot fungi cause a "reaction" or browning of the media under the fungus mats. O, negative reactors or brown-rot fungi; P, positive reactors or white-rot fungi.

The third letter refers to growth rate as mat diameters in centimeters. F, rapid, over 9 cm. in 7 days; I, moderately rapid, over 9 cm. in 14 days but less than 9 cm. in 7 days; M, medium, 5–9 cm. in 11 days; S, slow, 2–5 cm. in 14 days; V, very slow, less than 2 cm. in 14 days.

The numbers refer to microscopic structure. 1, clamps; 2, chlamydospores; 3, conidia; 4, oidia; 5, basidia; 6, basidiospores; 7, setae; 8, vesicular cells; 9, abnormal branching of hyphae; 10, all hyphae staining with eosin or erythrosin; 11, nonstaining hyphae present; 14, hyphae encrusted with crystals; 16, special structures or reactions described in descriptions of cultures.

In the key only those characters that are actually present are listed; characters not

listed should be considered absent or not readily observed. In separating the fungi with the same key pattern reference must be made to the cultural descriptions.

A–O–I–1–2, *Poria xantha;* *A–O–I–1–2–11, *Polyporus spraguei;* A–O–I–1–2–11, *Fomes pinicola;* A–O–M–1–2–10, *Polyporus balsameus;* A–O–M–1–2–11, *Trametes serialis;* A–O–M–1–2–11, *Poria sericeo-mollis;* *A–O–M–1–2–11, *Polyporus spraguei;*

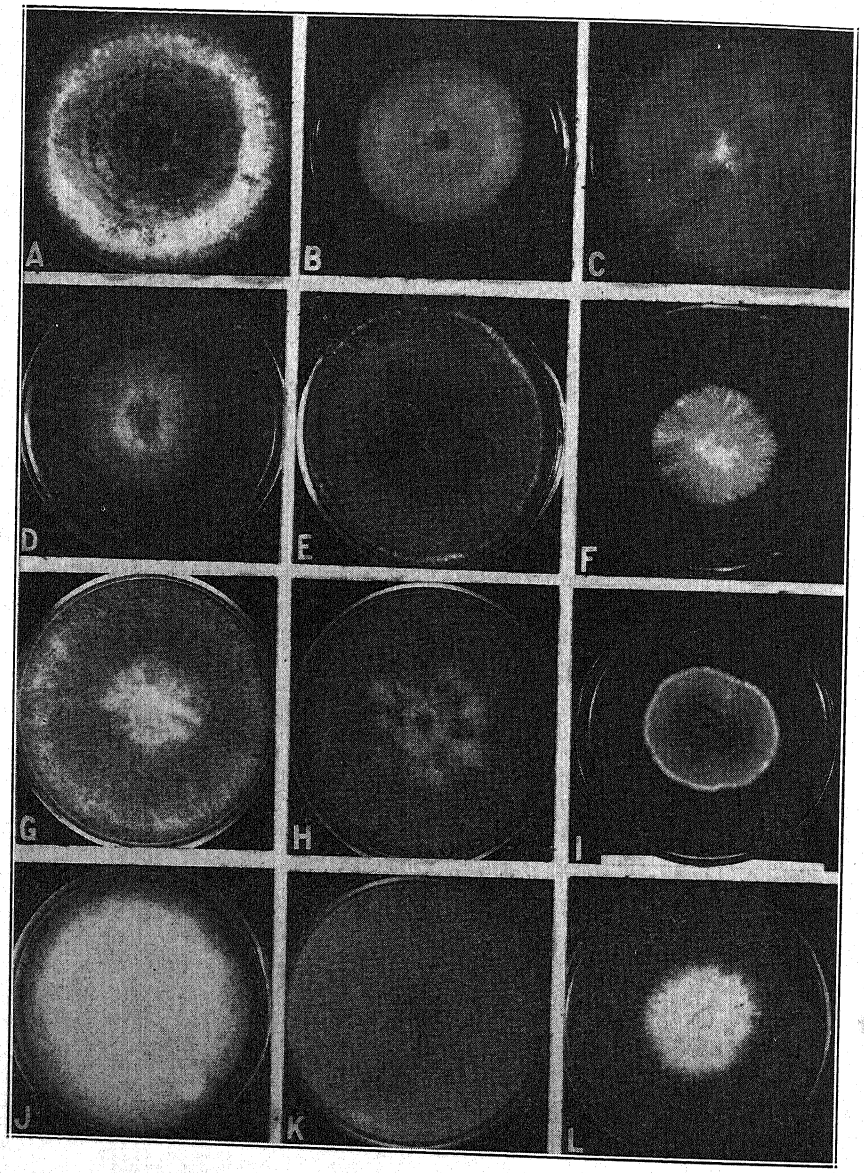



FIG. 6. Fourteen-day-old cultures of black cherry heartwood-inhabiting fungi. A, *Coniophora cerebella;* B and C, *Fomes pinicola;* D, *Polyporus balsameus;* E, *Polyporus schweinitzii;* F, *Polyporus subcartilagineus;* G and H, *Poria mutans;* I, *Poria prunicola;* J, *Poria sericeo-mollis;* K, *Poria xantha;* and L, *Trametes serialis.*

* Fungi whose cultural characteristics have been previously described in the oak decay study.

A–O–M–1–2–11, *Fomes pinicola;* *A–O–M–2–3–10, *Poria inflata;* *A–O–M–2–10, *Poria inflata;* A–O–S–1–2–10, *Polyporus subcartilagineus;* *A–P–F–1–11, *Polyporus versicolor;* *A–P–M–1–2–10–16, *Polyporus frondosus;* *A–P–M–1–2–10–16, *Hydnum erinaceus;* *A–P–M–2–5–6–11, *Polyporus berkeleyi;* B–O–F–1–11–16, *Coniophora cerebella;* B–O–I–1–2–11, *Poria xantha;* *B–P–F–1–11–16, *Stereum rameale;* *B–P–I–1–2–5–6–11–16, *Corticium lividum;* B–P–I–1–2–11, *Poria mutans;* C–P–M–11, *Poria prunicola;* *D–O–F–8–10, *Poria cocos;* D–P–M–11, *Poria prunicola;* *E–O–F–8–10, *Poria cocos;* *E–O–I–2–10, *Polyporus sulphureus;* E–O–M–1–2–10, *Polyporus balsameus;* E–O–M–1–2–11, *Poria sericeo-mollis;* *E–O–M–2–10, *Polyporus sulphureus.*

### CONIOPHORA CEREBELLA PERS.

(Fig. 6, A, and Fig. 7, A)

*Key Pattern.*—B–O–F–1–11–16.

*Growth Characteristics.*—Growth rapid, mat filling dish in 6 to 8 days, at first fragile, radiating in loose cottony strands, white to "Light Buff" (18); in 14 days mat variable, either loose-cottony, azonate, evenly "Light Buff" to "Cream Color," or with "Sayal Brown" and "Avellaneous" appressed patches alternating with raised, cottony "Light Buff" to "Cream Color" areas; surface mycelium either appressed cottony or nodulose or tufted.

*Hyphal Characteristics.*—Hyphae that stain with eosin 2–8 μ (10) in diameter, some of the hyphae with multiple clamps arranged in whorls around the septa, these easily demonstrated in young cultures, more difficult to find in 14-day-old mats; cross-walls also present independent of clamps; staining content in older hyphae often arranged in threads of varying diameter leaving an irregular nonstaining wall; in 14-day-old cultures yellow or brown hyphae with irregularly spaced knobs and side branches are occasionally found in darker portions of the mat.

*Type of Decay.*—Brown butt rot. Eight isolations.

*Remarks.*—Fritz (12) isolated a fungus from balsam fir rot (Type A), which was characterized by clamp connections in whorls. Her description of this fungus in culture indicates that it is probably *Coniophora cerebella. Coniophora cerebella* has also been isolated from balsam fir rot in New England.[6]

[6] Isolated by R. W. Davidson from rot sent in by P. Spaulding and J. R. Hansbrough.

### FOMES PINICOLA (SW. EX FR.) CKE.

(Fig. 6, B and C)

*Key Pattern.*—A–O–M–1–2–11 and A–O–I–1–2–11.

*Growth Characteristics.*—Isolates divided into two distinct growth forms (Fig. 6, B and C); the slow-growing strain in 14 days forming a mat 6–8 cm. in diameter, white, appressed, short-cottony or downy, zonate or azonate, occasionally somewhat pulverulent around the center; margin mostly white, even; fast-growing strain completely filling Petri dish, white or faintly white, azonate, appressed, fragile, thin matted-cottony.

*Hyphal Characteristics.*—Staining hyphae 2–5 μ in diameter, with many clamps; nonstaining fibrous hyphae 2–5 μ, smooth, no clamps; chlamydospores few or many, ellipsoid or very irregular in shape, 6–12 × 5–10 μ.

*Type of Decay.*—A brown top and trunk rot. 32 isolations.

*Remarks.*—Most of the isolates can be readily separated into the fast-growing strain or the slow-growing strain. Occasionally an isolate will be intermediate in respect to growth. The growth rate is not characteristic of isolates from any one host and cultures from cherry belong to both groups. The cultural characteristics of *Fomes pinicola* have been described by Campbell (3) and Mounce (14), both of whom found considerable variation in the growth rates of different isolates.

### POLYPORUS BALSAMEUS PK.

(Fig. 6, D, and Fig. 7, B)

*Key Pattern.*—A–O–M–1–2–10 and E–O–M–1–2–10.

*Growth Characteristics.*—Growth medium forming in 14 days a mat 5–7 cm. in diameter; mat white to agar-colored, often "Pinkish Buff" to "Cinnamon Buff" around the inoculum; short-cottony, fragile, homogeneous, azonate; margin faintly white, fimbriate.

*Hyphal Characteristics.*—Both submerged and superficial hyphae staining with eosin, up to 7 μ in diameter, clamps abundant; chlamydospores numerous, entire mat occasionally breaking down into a mass of spores, ellipsoid to barrel-shaped, 9–12 × 6–12 μ, with a hyaline wall and a yellow or brownish content.

*Type of Decay.*—Brown butt rot. Two isolations.

*Remarks.*—*Polyporus balsameus* is usually associated with a rot of balsam fir (13)

and other softwoods but has been collected on yellow birch and black cherry on the Allegheny National Forest. The fungus has been described in culture by Fritz (12) who noted the characteristic brownish chlamydospores.

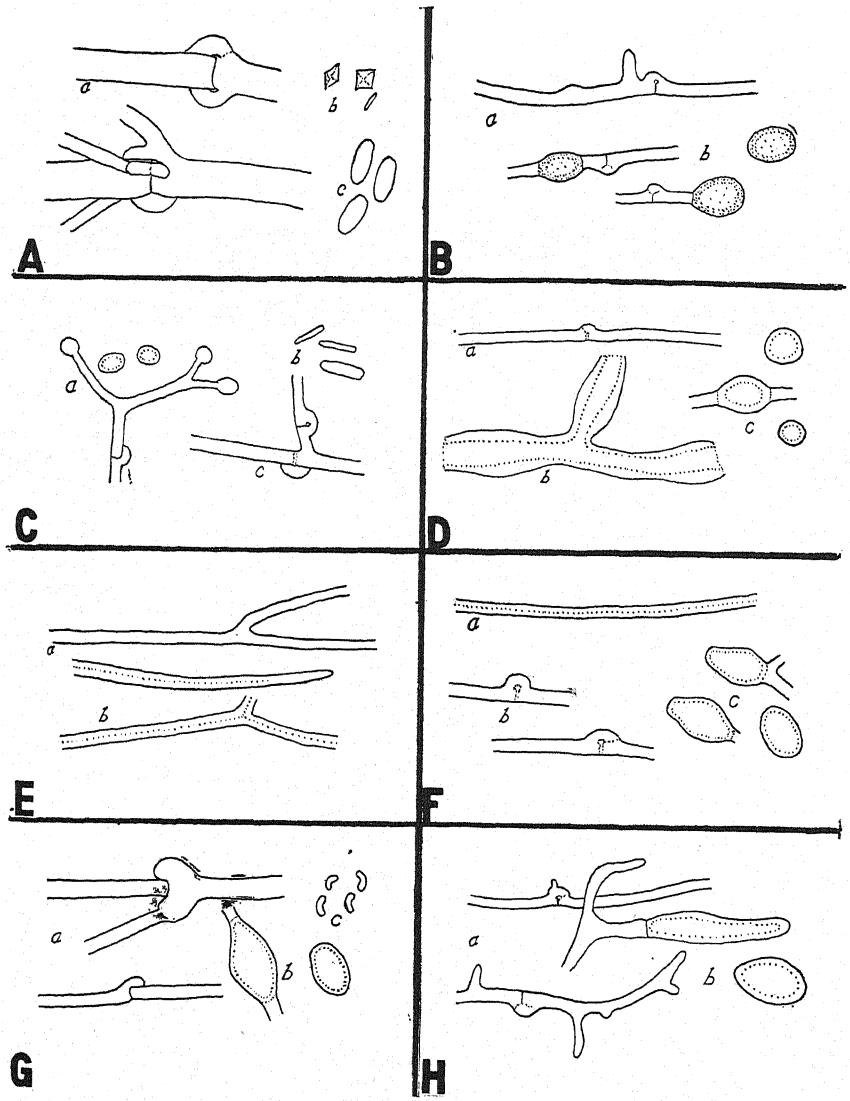



FIG. 7. A. *Coniophora cerebella:* a, hyphae with multiple clamps; b, crystals; c, oidia. B. *Polyporus balsameus:* a, thin-walled hypha; b, chlamydospores. C. *Polyporus subcartilagineus:* a, thin-walled hypha and chlamydospores; b, crystals; c, branched hypha with clamps. D. *Poria mutans:* a, thin-walled hypha; b, large irregular thick-walled hypha; c, chlamydospores. E. *P. prunicola:* a, thin-walled hypha; b, thick-walled hyphae. F. *P. serecio-mollis:* a, thick-walled hypha; b, thin-walled hyphae with clamps; c, chlamydospores. G. *P. xantha:* a, thin-walled hyphae with clamps; b, chlamydospores; c, basidiospores. H. *Trametes serialis:* a, thin-walled hyphae; b, chlamydospores.

POLYPORUS SUBCARTILAGINEUS OVERH.

(Fig. 6, F, and Fig. 7, C)

*Key Pattern.*—A–O–S–1–2–10.

*Growth Characteristics.*—Growth slow, forming in 14 days a mat 3.5–5 cm. in diam-

eter; mat white, loose-cottony with fine water droplets on aerial hyphae, very fragile, azonate; margin even, white.

*Hyphal Characteristics.*—All hyphae staining with eosin, 2–5 μ in diameter; clamps common; chlamydospores few, most abundant on inoculum of 14-day-old cultures, globose or ellipsoid, 5–10 μ in diameter.

*Type of Decay.*—Brown butt rot. 3 isolations.

*Remarks.*—This fungus is reported by Overholts (16) from *Picea* and *Prunus serotina* (Fig. 3, D). A similar fungus was also collected on *Liriodendron* by R. W. Davidson and K. D. Doak at Washington, D. C., which is culturally closely related to the fungus on black cherry. *Polyporus subcartilagineus* is a little-known species and no information is available as to its host preferences.

### PORIA MUTANS PK.

(Fig. 6, G and H, and Fig. 7, D)

*Key Pattern.*—B–P–I–1–2–11.

*Growth Characteristics.*—Growth moderately rapid, mat completely filling Petri dish in 14 days; central zone "Light Ochraceous Buff" to "Ochraceous Buff" and occasionally as deep as "Ochraceous Orange," irregular radiating or indefinite, appressed fine-woolly; rest of mat and marginal portions white or faintly white, appressed fragile usually with a belt of tufted mycelium near the edges of the dish.

*Hyphal Characteristics.*—Hyphae staining with eosin 2–4 μ in diameter, with many clamps spaced at short regular intervals on the hyphae, only occasionally branched at the clamps; fibrous hyphae at first with slightly thickened walls and staining content, gradually becoming empty with thick, even, or irregular walls, these hyphae prominent and characteristic 4–10 μ in diameter, with occasional cross-walls but no clamps; chlamydospores abundant or rare, mostly globose with a thick hyaline wall, up to 15 μ in diameter; colored hyphae from inoculum coated with fine crystalline material.

*Type of Decay.*—A white-piped heart and sap rot, usually associated with extensive trunk injuries. Eighteen isolations.

*Remarks.*—The relationship of this fungus to cultures of *Polyporus croceus* Pers. ex Fr. (10) has not been critically studied although they are very similar in appearance. It is possible that some of the *P. croceus* Pers. ex Fr. cases reported for oak are *Poria mutans*. So far as known the decays produced by these two fungi are also similar. *P. mutans* sporophores were found several times on old cherry logs which contained typical decay.

### PORIA PRUNICOLA (MURR.) SACC. AND TROTT.

(Fig. 6, I, and Fig. 7, E)

*File Pattern.*—C–P–M–11 and D–P–M–11.

*Growth Characteristics.*—Growth medium, forming in 14 days a mat 5–7 cm. in diameter; mat "Tawny Olive" and "Buckthorn Brown" with a narrow "Antimony Yellow" border 2–3 mm. wide next to the margin; moderately raised, azonate, with a fine, even, woolly surface, loose-felty in texture and peeling cleanly from the agar, not at all tough or leathery; margin proper white or slightly yellowish, narrow, 2–5 mm. wide, even, radiating short-cottony.

*Hyphal Characteristics.*—Hyphae staining with eosin 1–4 μ in diameter, branched with few cross-walls and no clamps, nonstaining hyphae yellow or dark brown, smooth or irregular, occasionally with slightly swollen spots, with cross-walls and branched, 1–4 (–5) μ in diameter.

*Type of Decay.*—A red mottled rot of the heartwood. In the incipient stage characterized by a darkening of the normal reddish heartwood. As the decay advances white pockets of completely decayed wood appear in the darkened heartwood. In the advanced stages the wood becomes soft with a white and dark reddish mottled appearance. Sixty-two decay cases.

*Remarks.*—No fruiting of the fungus was encountered on living infected black cherry and only one sporophore was collected on a down log of the species in the areas studied. Fruiting of *Poria prunicola* was very common on dead fire cherry. *Poria prunicola* sporophores cannot be separated on a morphological basis with certainty from *Fomes igniarius* var. *laevigatus* (Fr.) Overh. or *Poria spiculosa* Campb. & Davidson. These three species are distinct, however, in host relations and cultural characteristics (1, 5, 6).

### PORIA SERICEO-MOLLIS (ROM.) BAXTER

(Fig. 6, J, and Fig. 7, F)

*Key Pattern.*—A–O–M–1–2–11 and E–O–M–1–2–11.

*Growth Characteristics.*—Growth medium, forming in 14 days a mat 8 to 9 cm. in diameter; mat white or "Pale Pinkish Buff," fragile, fine-woolly to appressed cottony,

azonate or faintly zonate; margin appressed, colorless, even or fimbriate. Mats older than 14 days usually "Pale Pinkish Buff" to "Light Pinkish Cinnamon," cottony.

*Hyphal Characteristics.*—Hyphae staining with eosin 2–5 μ in diameter, with clamps; nonstaining fibrous hyphae 2–5 μ in diameter, colorless; chlamydospores few or many, lemon-shaped to globose or ellipsoid, 10–16 × 7–12 μ; basidiospores commonly produced in pores, which develop on the upper part of slant on tube cultures, short cylindric 5–7 × 3–4 μ.

*Type of Decay.*—Brown cubical rot. Ten isolations.

*Remarks.*—*Poria sericeo-mollis* sporophores have been noted on badly decayed logs of black cherry and also on large open wounds on living trees. Isolations of this fungus were most common from rot associated with large wounds such as those caused by the splitting of forked stems. The interpretation of the species is that given by Overholts (17).

### PORIA XANTHA (FR. EX LIND) CKE.

(Fig. 6, K, and Fig. 7, G)

*Key Pattern.*—A–O–I–1–2–11 and B–O–I–1–2–11.

*Growth Characteristics.*—Growth moderately rapid, mat completely filling a 9-cm. Petri dish in 14 days; mat white or "Sulphur yellow," azonate, thin, fragile, appressed around the inoculum with scant aerial mycelium, fine-woolly near margin and often forming considerable loose-cottony aerial mycelium against the sides of the dish.

*Hyphal Characteristics.*—Hyphae staining with eosin 2–8 μ in diameter, clamps common, often double; the larger hyphae finally become empty with a thick hyaline wall or develop the hyaline wall and retain a narrow, irregular lumen, which stains with eosin; chlamydospores, few or many, globose to lemon-shape, with a narrow hyaline wall; an occasional isolate fruits in culture producing allantoid basidiospores 4–6 × 1.5–2.5 μ.

*Type of Decay.*—Brown cubical rot. One isolation from a badly rotted stump upon which the fungus was fruiting.

*Remarks.*—*Poria xantha* is common on softwoods (17); often producing decay in structural timbers. Isolates from black cherry, white pine, and hemlock all had the same cultural characteristics.

### TRAMETES SERIALIS FR.

(Fig. 6, L, and Fig. 7, H)

*Key Pattern.*—A–O–M–1–2–11.

*Growth Characteristics.*—Growth medium, forming in 14 days a mat 6–7 cm. in diameter; mat usually with a tough, white, appressed, occasionally considerably thickened central zone and a wide, faintly white or colorless, appressed, thin, fragile marginal zone; margin proper colorless, appressed, fimbriate. Central zone at times with radiating irregularly spaced ridges and usually with very little loose surface mycelium.

*Hyphal Characteristics.*—Hyphae staining with eosin 2–5 μ, clamps abundant; fibrous nonstaining hyphae 2–4 μ diameter; chlamydospores few or many, often irregular in shape, 9–18 × 7–10 μ; immature basidia also formed in some isolations in 14 days.

*Type of Rot.*—Brown rot. Five isolations.

*Remarks.*—This is not the fungus described from lumber by Cartwright and Findlay (7) and not the same as the culture mentioned by Baxter (2) in his discussion of *T. serialis* and closely related forms. In northern Pennsylvania this form occurs also on other hardwoods and on hemlock.

## SUMMARY

A study of cull caused by decay was made in 3 merchantable stands of black cherry on the Allegheny National Forest. Numerous infections, mostly in the butts, were present in 52-year-old trees; these, however, caused only 2.3 per cent cull based on board-foot volume. In two old-growth stands (116 and 120 years old), one of almost pure dense cherry and the other open-grown mixed hardwoods, cull from top or trunk infections was more important than butt rot. In the open-grown 120-year-old trees there was 6.1 per cent cull and in the pure dense stand there was 11.3 per cent cull from decay.

The most important butt rots were caused by *Polyporus spraguei, P. berkeleyi,* and *Coniophora cerebella.* The most important trunk rots were those caused by *Poria prunicola, P. mutans, Fomes pinicola* and *Polyporus*

*sulphureus.* These trunk rotters entered the trees principally through large branch stubs, except *Poria mutans,* which was usually associated with large wounds.

Most of the rots of black cherry present no external evidence of their presence, except *Fomes pinicola,* which formed conks on half of the trees infected by it.

Twenty-two species of decay-producing fungi were isolated from 212 rot infections in black cherry. Those most frequently isolated were: *Poria prunicola* (62 times), *Fomes pinicola* (32), *Polyporus sulphureus* (28), *Poria mutans* (18), *Polyporus spraguei* (14), *Poria sericeo-mollis* (10), *Polyporus berkeleyi* (9), *Coniophora cerebella* (8), *Trametes serialis* (5), and *Poria inflata* (5).

Good quality black cherry can be grown to large sawlog size without excessive cull from decay. Management to increase diameter growth after the early height growth is attained should hold down the amount of cull from decay if the trunks of the reserved trees are not injured severely.

## LITERATURE CITED


1. BAXTER, D. V. Some resupinate polypores from the region of the Great Lakes. V. Papers Mich. Acad. Sci., Arts, and Letters 19: 305–332. 1934.
2. ————. Some resupinate polypores from the region of the Great Lakes. XIII. Papers Mich. Acad. Sci., Arts, and Letters 27: 139–161. 1942.
3. CAMPBELL, W. A. The cultural characteristics of the species of *Fomes.* Bull. Torr. Bot. Club 65: 31–69. 1938.
4. ————, and ROSS W. DAVIDSON. Top rot in glaze-damaged black cherry and sugar maple on the Allegheny Plateau. Jour. For. 38: 963–965. 1940.
5. ————, and ————. A species of *Poria* causing rot and cankers of hickory and oaks. Mycologia 34: 17–26. 1942.
6. ————, and ————. Cankers and decay of yellow birch, associated with *Fomes igniarius* var. *laevigatus.* Jour. For. 39: 559–560. 1941.
7. CARTWRIGHT, K. G., and W. P. K. FINDLAY. The diagnosis of decay in timber. Empire For. Jour. 9: 190–203. 1930.
8. CHILDS, T. W. Variability of *Polyporus schweinitzii* in culture. Phytopath. 27: 29–50. 1937.
9. DAVIDSON, R. W., W. A. CAMPBELL and D. J. BLAISDELL. Differentiation of wood-decaying fungi by their reaction on gallic or tannic acid medium. Jour. Agr. Res. [U.S.] 57: 683–695. 1938.
10. ————, ————, and D. B. VAUGHN. Fungi causing decay of living oaks in the eastern United States and their cultural identification. U. S. Dept. Agr. Tech. Bull. 785, 65 pp. 1942.
11. DOWNS, ALBERT A. Glaze damage in the birch-beech-maple-hemlock type of Pennsylvania and New York. Jour. For. 36: 63–70. 1938.
12. FRITZ, CLARA W. Cultural criteria for the distinction of wood-destroying fungi. Proc. and Trans. Roy. Soc. Canada 17: 191–288. 1923.
13. HUBERT, E. E. A butt rot of balsam fir caused by *Polyporus balsameus* Pk. Phytopath. 19: 725–732. 1932.
14. MOUNCE, IRENE. Studies in Forest Pathology II. The biology of *Fomes pinicola* (Sw.) Cooke. Dom. Canada Bull. Dept. Agr. (n.s.) 111, 74 pp. 1929.
15. OSTROM, C. E. Clear cutting of young northern hardwood stands. Jour. For. 36: 44–49. 1938.
16. OVERHOLTS, L. O. New species of Polyporaceae. Mycologia 33: 90–102. 1941.
17. ————. The Polyporaceae of Pennsylvania, The genus *Poria.* Pa. Agr. Exp. Stat. Tech. Bull. 418, 64 pp. 1942.
18. RIDGWAY, R. Color standards and color nomenclature. 43 pp. and 53 colored plates. (Washington, D. C.) 1912.

# THE FUNGUS CAUSING THE SO-CALLED "SEPTORIA LEAF-SPOT DISEASE" OF RASPBERRY

J. B. DEMAREE AND MARGUERITE S. WILCOX[1]

(Accepted for publication April 1, 1943)

Infection characterized by small, angular to circular, grayish lesions on living leaves of raspberry, commonly known as Septoria leaf-spot disease, is prevalent in most regions in the United States. It is the most destructive fungus disease of raspberry in the Middle Atlantic States and westward to Arkansas. A similar disease, caused by *Septoria rubi* West., attacks the *Eubatus* group of *Rubus* and is prevalent in almost every region where members of this group are found.

For almost a century mycologists and plant pathologists generally have considered *Septoria rubi* or one of its described varieties to be responsible for the disease in all species of *Rubus*. In 1921 an ascogenous stage of the fungus, *Mycosphaerella rubi*, was named and described by Roark (15) from overwintered leaves of both raspberry and blackberry.

In 1939 the writers observed that a *Sphaerulina* occurred abundantly on overwintered raspberry leaves at Beltsville, Maryland, and found that it was a stage in the life cycle of the common raspberry leaf-spot fungus, although it failed to infect leaves of blackberry and dewberry. These observations led to a more extensive study of the fungi causing the so-called Septoria leaf spot of *Rubus* spp., and the findings are herein reported. The evidence indicates that the fungus attacking raspberry and having a *Sphaerulina* perfect stage attacks neither blackberry nor dewberry.

## REVIEW OF LITERATURE

Reference in literature to the raspberry fungus is very meager. In 1885 Ellis and Morgan (in a paper by Ellis and Everhart (8)) published as new the species *Cylindrosporium rubi* found on living leaves of the black raspberry (*Rubus strigosus* Michx.), from a collection apparently made by J. J. Brown of Wisconsin. Their description of the disease agrees in general with observations made by the writers of the raspberry disease and fungus now under consideration. Furthermore, a specimen in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, which is undoubtedly a part of the type collection of *C. rubi* collected by J. J. Brown in Wisconsin in 1885, was examined by the writers, who believe it to be identical in every respect with the common raspberry fungus heretofore referred to as *Septoria rubi* West.

Zundel (23), in 1942, reported *Cylindrosporium rubi* as causing widespread damage to red raspberries in Pennsylvania.

[1] Senior pathologist and assistant pathologist, respectively, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, Bureau of Plant Industry Station, Beltsville, Maryland.

It is not clear just when *Septoria rubi* was first reported as associated with a raspberry disease similar to that of the blackberry. Galloway (9) reported upon "experiments in the treatment of *Septoria* of raspberry and blackberry" as early as 1891. In Rabenhorst's Krypogamen Flora, Allescher (1), in 1901, lists raspberry, in addition to blackberry and dewberry, as a host of *S. rubi.* Bennett (3) mentioned that "raspberry, dewberry, and blackberry are attacked by a rather common type of leaf spot." Colby, Anderson, and Flint (5) wrote: "On raspberry leaves these spots soon turn to ashen-gray in the center. On blackberry and dewberry leaves the edge of the spots has a zone of purple tissue and the center is light brown to tan in color. . . . The disease is caused by a fungus, *Mycosphaerella rubi.* It is more commonly known under the old name *Septoria rubi* which was applied before the perfect stage was known." Dodge and Wilcox (7) stated that "leaves of red raspberry are frequently attacked by the same fungus that causes leaf spot of dewberry." Hesler and Whetzel (10) reported that "the common leaf spot of blackberry affects also raspberry and dewberry." Roark, in the opening statement of his paper describing the perfect stage of the Rubus *Septoria,* said: "The fungus formerly known as *Septoria rubi* Westendorp causes leaf spots on many species of *Rubus.* . . ."

The blackberry leaf-spot fungus was given the name *Septoria rubi* by Westendorp (19) in 1854, when issued in Westendorp and Wallay's Herbier Cryptogamique Belge, Fasc. 19–20, No. 938, and accompanied by label with description. He gave leaves of *ronce* (briar, bramble) as the host. The pycnospores (*sporidies*) were described as cylindrical, rounded at both ends, with 3 to 5 segments (sporules) very difficult to see, 1/100 to 1/200 mm. long. There seems to be some gap in the early taxonomic history of the fungus. On the label of the specimen two synonyms are given as follows: "*Sphaeria rubi* Duby, Bot. Gall II, p. 712 ?—*Depazea rubi* West. et Vanhaes, Cat. Crypt., No. 80." The question mark after "p. 712" may mean that Westendorp was uncertain as to the synonymy. The writers have not been able to find in literature and exsiccata available for examination any reference to *Depazea rubi.*

Kickx (12) in 1867 (p. 453) gave a fuller description of the fruiting body and the leaf manifestations of the fungus, but used Westendorp's description and measurements of the pycnospores.

Saccardo's (17) description of the fungus and the spots on the leaves is similar to the descriptions by Westendorp and by Kickx, except that he recorded the pycnospore dimensions as 40 to 55 μ long and 1.5 μ wide.

Berkeley and Curtis (4), apparently not knowing of Westendorp's *Septoria rubi,* gave the same name in 1878 to a fungus on Rubus leaves collected along the Santee River in South Carolina. Of course Westendorp's name has priority over *Septoria rubi* Berk. and Curt.

Several other references to *Septoria* on *Rubus* are recorded in literature; 2 forms are designated as distinct species and others as varieties of *S. rubi.* Descriptions of these are either meager, or based upon the color of the

resulting lesions, or the pycnospores are shorter than the usual concept of *S. rubi* West. In the opinion of the writers, length of the pycnospores alone cannot always be used as a basis for separating species or variants. They have found that the pycnospores, especially those on raspberry, vary considerably in the same pycnidium. Those formed immediately after maturity of the pycnidia are longer and more normal than those at the end of the period of pycnospore formation. Old pycnidia, once spent or exhausted, will contain a few short and otherwise abnormal pycnospores. It is thought that some descriptions of *Septoria* on *Rubus* have been made from material too old for satisfactory diagnosis.

*Septoria comitata* J. J. Davis was described from Caeoma-infected leaves of *Rubus allegheniensis*. The description does not indicate the fungus to be different from *S. rubi* West. Davis (6) made the following notation in his description: "While it is possible that this is a form of *S. rubi* West., modified by the form of the substratum, it is being kept separate under the name *Septoria comitata* n. sp. ad interim." The following varieties of *Septoria rubi* West. have been described as occurring on either blackberries or dewberries: Var. *pallida* Ell. and Holway; var. *brevispora* Sacc.; var. *saxatiles* Allescher; and var. *asiatica* Bub. There also has been described var. *alba* Pk. of *Septoria rubi* Berk. and Curt. The first two were separated because of their short pycnospores. The writers examined a portion of the original collection of Saccardo's variety *brevispora* kindly sent to them by H. D. House, curator of the New York State Museum. Their measurement of pycnospores from this material showed them to vary from 22–48 μ by 1.6–3 μ. This is not greatly out of line with the normal dimensions of *S. rubi* West. in blackberry.

It seems that the fungus also has been designated as *Ascochyta rubi* Lascher (13), *Rhabdospora ramealis* (Desm. and Rob.) Sacc. (18), and *Sphaerella ligea* Sacc. (16).

In 1937, Zeller (21), after examining specimens of *Septoria*-infected leaves of *Rubus* from various localities, concluded that there are 2 Septoria leaf-spot diseases of *Rubus* in the United States and reported: "The materials indicated that the leaf spots from the Pacific Northwest, Wisconsin, Durham, North Carolina, and England are essentially alike and constitute the usual concept of *Septoria rubi* West. The leaf-spot material sent from Beltsville, Maryland, and Willard, N. C., however, had a different appearance." He further reported that the Beltsville material proved to be a fungus first described by Saccardo as *S. rubi* West. var. *brevispora* from material on leaves of *Rubus hispidus* collected by C. H. Peck at New Chatham, New York. Zeller believed that there were so many distinct physiological and morphological differences between *S. rubi* West. and var. *brevispora* Sacc. that one could not be considered a variety of the other, and he, therefore, raised Saccardo's variety to specific rank and proposed the new combination *S. brevispora* (Sacc.) Zeller. Later, learning that the binominal *S. brevispora* was preoccupied, he applied the new name *S. darrowi* (22).

Zeller was not very specific as to the host on which he found his *Septoria darrowi* (*S. brevispora*), but considered it broadly as a fungus parasitic on the genus *Rubus*. He referred to what one would expect to be his type specimens as the "Beltsville, Maryland, material." That could have been one of several species of blackberry, dewberry, or raspberry. He, however, illustrated his fungus by a drawing of a pycnidium and pycnospores from a raspberry leaf and showed for comparison a drawing of *S. rubi* in Himalaya blackberry (*Rubus procerus*). He described his fungus as characterized by small pycnidia (27–35 μ high and 46–60 μ broad) with pycnospores 15–30 × 1.8–3.4 μ. Presumably, he was describing the raspberry fungus. If so, the name *Cylindrosporium rubi* Ell. and Morg. should have priority over Zeller's *S. darrowi* in absence of proof of their dissimilarity.

The short pycnospores recorded by Zeller could have been attributed to the condition of his specimens. If his specimens were collected in autumn and the normal pycnospores had been discharged and washed or weathered out, then he would have had for measuring only the abnormal, short, blunt, 1- to 3-septate pycnospores in the spent pycnidia.

From the descriptions it would appear that *Septoria rubi* Berk. and Curt., *S. comitata* J. J. Davis, and all varieties of *S. rubi* should be considered as synonyms of *S. rubi* West.

There seems to be some significance in the fact that all species of Septoria on brambles have been recorded as pathogens in "*Rubus*" or in some blackberry species; in no case in any original description has the raspberry been mentioned specifically as a host.

## THE RASPBERRY PATHOGEN

### The Pycnidial Stage

The raspberry leaf-spot fungus is parasitic in living leaves and in the thin cortical layer of canes, and forms pycnidia and perithecia in fallen leaves during fall and winter.

On the green leaves, the immature fruiting bodies show faintly under the upper epidermis and within the boundary of small greenish-black spots (Fig. 1, A) about as soon as the spots are perceptible. Development of the pycnidia proceeds rapidly. They mature and pycnospores are produced and discharged before the fungus-invaded tissues are completely necrotic, as is evidenced by a color change from greenish-black to gray (Fig. 1, B).

The pycnidia are subglobose to ovoid, average about 70 μ high and 80 μ wide, and lie within the palisade region of the leaf structure (Fig. 1, C) with their base extending well into the spongy parenchyma. In the early stages of its development, before the initiation of pycnospores, the pycnidial wall consists of a thin layer of closely interwoven hyphae. At maturity the base and side comprise 1 to 3 layers of thin-walled cells, and the top is covered with 1 layer, devoid of any ostiole structure. Apparently, the pressure exerted from within by the production of a large quantity of pycnospores is sufficient to rupture the pycnidial covering and the over-lying epidermis.

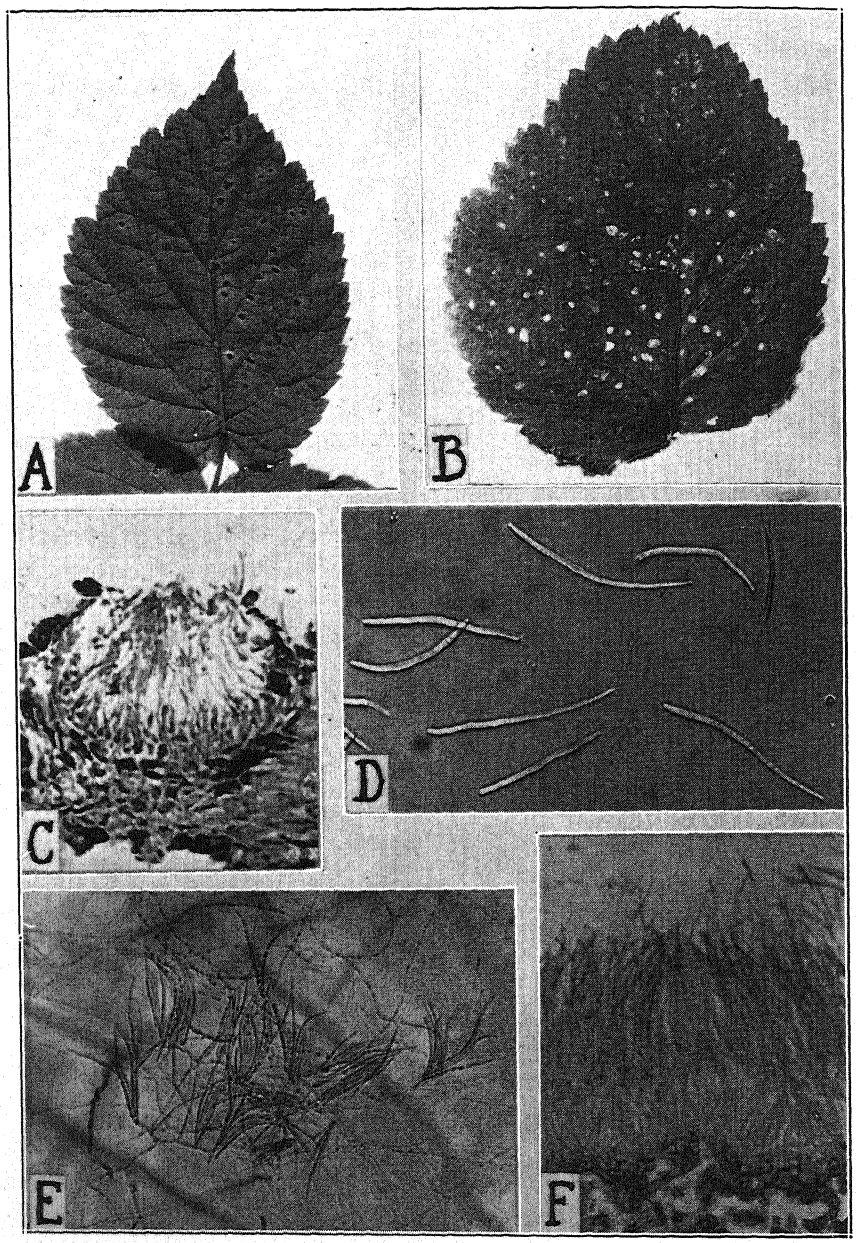



FIG. 1. A. Young raspberry leaflet showing early stage of the disease, when the pycnidia are discharging pycnospores. B. Leaflet showing older and more conspicuous stage of spotting, after the pycnospores have been discharged. C. Pycnidium from a living leaf. ×350. D. Pycnospores from a leaf pycnidium. ×375. E. Conidia formed on hyphae in young colony on corn-meal agar. ×125. F. Heavy production of conidia from a sporogenous layer in an old corn-meal agar culture. ×350.

The pycnospores are discharged as an aggregate, with the shape of a horn or cylinder, and having a diameter about equal to that of the fruiting body, which becomes hard when dry. Dew or rain dissolves the matrix cementing the pycnospores. The pycnospores may later be washed away, leaving the empty pycnidium surrounded by a ring of ruptured epidermis, which then appears as a tiny crater.

The pycnospores develop from short sterigmata growing from the sides and base of the pycnidium. The pycnospores are elongate, usually curved, slightly obclavate, thickest about one-third the distance from the base, and tapering toward the apex (Fig. 1, D). They are hyaline, granular, 3- to 9-septate, and average 55 μ long and 3.5 μ wide. Although the length varies from 32 to 86 μ, the great majority of the pycnospores fall within the range of 40 to 60 μ. Measurement of width of pycnospores, made through region of greatest dimension, varies from 3.0 to 4.8 μ. Germination takes place terminally and laterally.

The fungus commonly infects the canes of the raspberry and usually shows only as scattered pycnidia on the surface of the lower portion of the canes, or infrequently causes discolored or necrotic areas in the cortical tissues. Because cane infections do not always cause conspicuous lesions, infected canes probably occur more commonly than is ordinarily suspected; in fact, pycnidia frequently were observed on canes of raspberry varieties whose leaves are susceptible to infection. The fungus on the canes was demonstrated by inoculation experiments to be identical with the fungus on the leaves (see page 999). This fungus is thought to be the same as described by Ellis (published by George Martin (14)) from raspberry canes and named *Rhabdospora rubi*. The pycnidia, although showing on the canes during late fall and winter, do not sporulate until May or June and are undoubtedly an important source from which new spring leaves become infected. Pycnidia on planting stock may serve to distribute the fungus from one locality to another (Fig. 2, F).

The cane pycnidia are subepidermal and partly imbedded within the cortex (Fig. 2, G). They vary from subglobose-ovoid to lenticular, and have a broad ostiole through which the conidia are discharged in the form of a waxy cirrus. These fruiting bodies are more typical of the usual concept of a pycnidium than those that develop in living raspberry leaves.

The pycnospores in most respects resemble those formed during the summer in leaves. The cane form is shorter, averaging 40.8 μ long, but has a width equal to those in the leaves. The most significant difference noted is that there are fewer showing the obclavate shape. Most of them are uniformly cylindrical from the base to the tapering apical cell.

Soon after the infected leaves fall, winter pycnidia begin to develop near the old lesions. These show on both surfaces but in greater number on the underside, where they are concealed by the dense tomentum, unless it is wet. These pycnidia may be found at any time from late autumn to spring. They are at first subepidermal but emerge later through the epidermis and appear

to be quite superficial. They are black, subglobose to urn-shape and measure about 80–121 × 65–101 μ, averaging 94 to 82 μ (Fig. 2, A, a). The side of the pycnidial wall is composed of 2 to 4 layers of thick-walled cells.

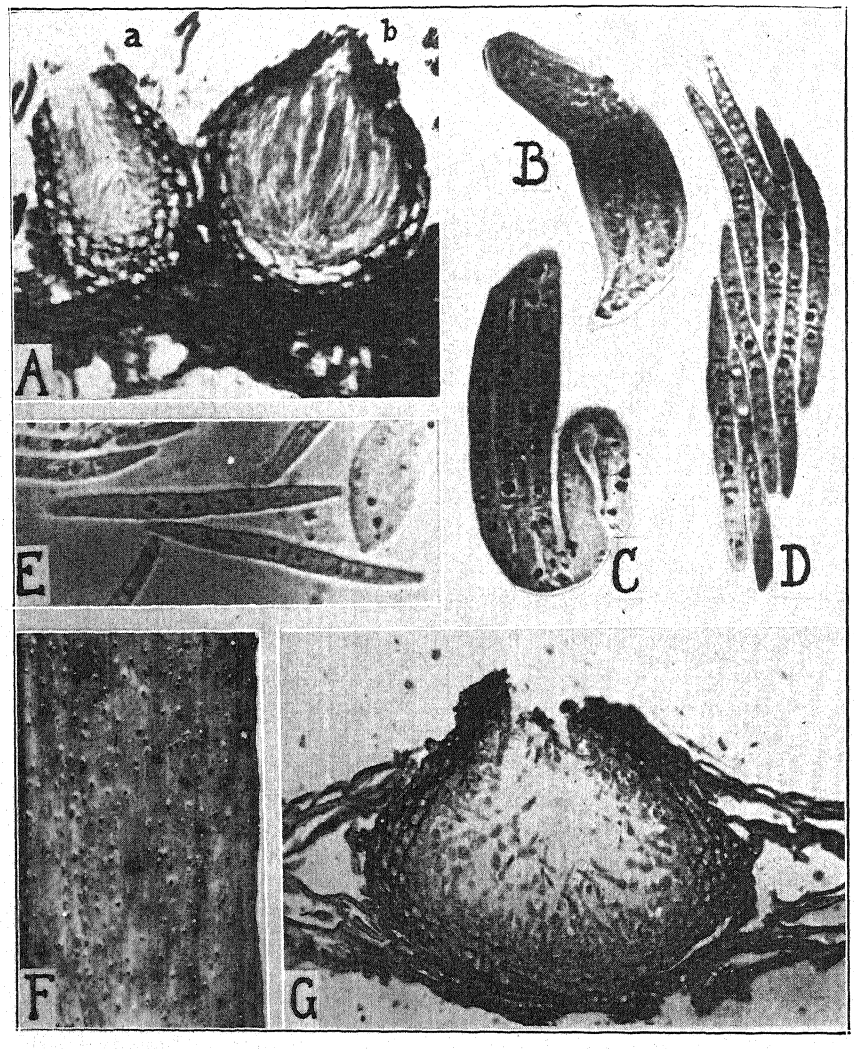



FIG. 2. Winter stage of the raspberry leaf spot fungus. A. A pycnidium (a) and a perithecium (b) of the *Sphaerulina* on an overwintered leaf. × 350. B. An ascus as usually seen in freshly mounted specimens. C. Developing ascospores being discharged from a ruptured ascus, but still retained by an inner ascus membrane. × 800. D. Fully developed ascospores immediately after being liberated from an ascus. × 800. E. Ascospores showing characteristic septation. × 800. F. Raspberry cane showing scattered distribution of the winter pycnidia. × 4. G. A winter pycnidium from a cane. × 350.

Stained sections do not show clearly the presence of an ostiole, and the weight of evidence indicates that an ostiole is lacking.

The winter pycnospores are in all essentials like those produced on green leaves. The cultural characters of the overwintering fungus are identical with those of the summer form. The presence of both pycnidia and perithecia on overwintered, diseased leaves, with an apparent cellular connection (Fig. 2, A, a and b) strongly suggests genetic relationship. Proof of such relationship by inoculation was not attempted.

### THE ASCOGENOUS STAGE

In the spring of 1939 a search was made for the ascogenous stage of the common Septoria on overwintered leaves of blackberry, dewberry, and raspberry. According to Roark (15) the perfect stage is a *Mycosphaerella*. No fungus resembling that described by Roark was found on any leaves examined, although perithecia of the *Sphaerulina* type were commonly observed on raspberry leaves. This fungus was assumed to be some saphrophyte, hence no further study of it was then made.

The following spring another search was made for the *Mycosphaerella* on *Rubus*. *Sphaerulina* was again plentiful on overwintered raspberry leaves. Two collections of dewberry leaves were made that bore perithecia of the *Mycosphaerella* type (cultures from these perithecia proved later not to be parasitic on *Rubus*); and of several collections of overwintered leaves of blackberry none had perithecia of any kind.

Ascospores from perithecia of the Sphaerulina type of the raspberry fungus were isolated and grown on corn-meal agar; the resulting growth of

TABLE 1.—*Results of direct and cross inoculation of leaves of raspberry, dewberry, and blackberry with pathogens isolated from each form*

| Source of cultures used for inoculum | Plant inoculated | Number of plants inoculated | Number of leaves inoculated | Number of leaves infected | Number of lesions appearing |
|---|---|---|---|---|---|
| Pycnospores from living raspberry leaf | Raspberry | 10 | 85 | 75 | 22,350 |
| | Dewberry | 11 | 99 | 0 | 0 |
| | Blackberry | 11 | 90 | 0 | 0 |
| *Sphaerulina* ascospores from overwintered raspberry leaf | Raspberry | 10 | 132 | 121 | 18,300 |
| | Dewberry | 4 | 67 | 0 | 0 |
| | Blackberry | 3 | 24 | 0 | 0 |
| Pycnospores from living dewberry leaf | Dewberry | 5 | 54 | 35 | 1,490 |
| | Blackberry | 10 | 104 | 79 | 450 |
| | Raspberry | 5 | 47 | 0 | 0 |
| Pycnospores from living blackberry leaf | Blackberry | 11 | 79 | 53 | 1,180 |
| | Dewberry | 7 | 51 | 19 | 275 |
| | Raspberry | 7 | 62 | 0[a] | 0[a] |

[a] Two plants infected. See text, p. 999.

hyphae and conidia resembled the fungus previously isolated by using pycnospores of the leaf-spot fungus from living raspberry leaves. A water suspension of the conidia from ascospore isolates was applied to the foliage of disease-free potted raspberry plants in a greenhouse. In about 2 weeks lesions appeared on the inoculated leaves. In these lesions occurred fruit-

ing bodies and pycnospores similar to those of the fungus naturally present on raspberry leaves. During the following 2 years, numerous successful inoculations were made on raspberry leaves with conidia from cultures originating from single-ascospore isolations of the raspberry *Sphaerulina*. Parallel inoculations on dewberry and blackberry leaves were negative. The results of these inoculations are given in table 1.

## The Perithecia

Perithecia form in late fall; asci develop only on resumption of warm spring weather. The fungus, somewhat sensitive to weather conditions, does not complete its growth cycle if weather is too hot or too dry. Perithecia are largely superficial, with bases embedded in spongy parenchyma, and protrude through broken epidermis. They are black, ovate to conical, slightly papillate, 88–140 μ high, and 86–120 μ wide (Fig. 2, A, b). The perithecial wall is thin, comprising 2 to 3 layers of cells.

Development of the asci and ascospores was observed over a period of several hours after mounting in a drop of tap water inverted over a Van Tieghem cell. Usually, when asci were first separated from the perithecium, they appeared immature (Fig. 2, B), with homogenous and granular contents; but, within 2 hours, faint lines representing the 8 ascospores began to show (Fig. 2, C). The development of the ascospores continued slowly until their form was seen clearly. The ascospores at this stage were packed within the ascus without any apparent order. Simultaneously, with the development of ascospores, the ascus slowly distended 20 to 30 μ in the direction of the long axis and at the same time the spores changed position to fill this space (Fig. 2, C). What happens to the ascus wall is not clearly understood, but apparently it ruptures horizontally and the tip is pushed upward or to one side by the pressure of the developing spores. An inner membrane, or spore-enclosing, wall disappears and the mature spores drift apart (Fig. 2, D). No indication of their forceful discharge has been observed.

Asci are clavate-cylindrical, with obtuse or somewhat pointed apex; 44.8 μ to 70.4 μ long by 9.6 μ to 15.0 μ wide. The ascus wall is 2–3 μ thick, sometimes apparently thicker at free end; no paraphyses were observed. The ascospores are hyaline, granular, cylindrical, ends somewhat pointed but a little more so at apex, slightly curved, usually 4-celled, less frequently 6–8-celled, 32–57 μ long by 3.5 to 5.8 μ wide (Fig. 2, D and E).

The spores germinate within 1 or 2 hours after liberation from an ascus. Usually a germ tube grows from the free end of each terminal cell. Less frequently the intermediate cells germinate; and when they do, the germ tube emerges near a septum.

The writers made a search for perithecia on overwintered leaves of various form of *Rubus* for 3 successive years. In addition to examining collections taken from different localities, they collected in the autumn spotted leaves of blackberry, dewberry, and raspberry at Beltsville, Maryland. A

portion of these collections were left in the field with only enough protection to prevent scattering by the wind, and marked so they could be found for examination the following spring. Other portions of the collection were placed in floorless wire cages, so that the leaves would be in contact with the soil. At various times during the winter and spring, specimens from the field or cages were transferred to a greenhouse or moist chamber in the laboratory. The *Sphaerulina* was found on raspberry leaves each year without difficulty.

Only infrequently have perithecia of any kind been observed on overwintered blackberry and dewberry leaves. Perithecia of *Mycosphaerella* type were observed on a few collections taken near Beltsville, Md., and near Rose Hill, Willard, and Raleigh, N. C. The size of the asci and ascospores, and character of the growth on artificial media indicated that 3 groups or species were represented. One type, collected in 1941 on dewberry at Rose Hill, N. C., and in 1942 at Beltsville, Md., produced small, hyaline, 1-septate ascospores, the 2 cells being of unequal size. The spore measurements varied from 6.8 to 12.0 μ long by 2.6 to 3.8 μ wide. Twenty-seven single-spore isolates were made from this form. The colonies on artificial media did not sporulate and were otherwise unlike any fungus isolated from living *Rubus* leaves.

A second form, also bearing ascospores with cells of unequal size, was collected on overwintered dewberry leaves at Beltsville. These spores ranged from 12.0 to 16.0 μ long, by 3.2 to 3.5 μ wide. Thirty-two single-spore isolations were made from the 2 collections. The appearance of this fungus on corn-meal agar somewhat resembled the dewberry leaf-spot fungus. Black, conical, ostiolate pycnidia were formed that extruded masses of cream-colored filiform pycnospores measuring 17.8 to 27.6 μ long and 1.6 to 2.5 μ wide, or about half the length of the pycnospores of the dewberry Septoria. Several attempts were made to infect dewberry and blackberry leaves with this ascospore isolate. Fourteen Lucretia dewberry and 2 Lawton blackberry plants were inoculated. No infection developed. The fungus was considered non-parasitic on the 2 *Rubus* species inoculated and different from *Septoria rubi.*

The form most frequently present on overwintered leaves was collected on dewberry at Beltsville in 1940 and 1942, also at Rose Hill, N. C., in 1941, and on blackberry and dewberry at Raleigh, N. C., in 1942. Ascospores were hyaline, 1-septate, the 2 ascospore cells being about equal in size. Ascospores were 14.4 to 22.0 by 2.8 to 3.5 μ in dimensions. The spores of this fungus are only slightly smaller than the measurements reported by Roark (15) for *Mycosphaerella rubi;* and are apparently identical with those of the single specimen filed in the Mycological Collections of the Bureau of Plant Industry, labeled *Mycosphaerella rubi* (West.) Roark. This specimen was determined by F. A. Wolf from a collection made at Raleigh, N. C., in 1924.

Eighty single-spore isolations were obtained by the writers from the collections taken from the 3 localities mentioned above. The resulting colonies resembled somewhat those of *Septoria rubi,* but grew more rapidly and produced neither conidia nor pycnospores. It is believed, therefore, that this fungus is not the ascogenous stage of *S. rubi* West. The fungus the writers do consider to be *S. rubi* West., which attacks living leaves of the *Eubatus* group of *Rubus,* has been isolated from collections made from various parts of the United States, and in every case has produced either conidia or pycnospores on corn-meal agar.

In Roark's (15) paper on the Septoria leaf spot of *Rubus* the claim is made that the Septoria occurring on *Rubus* is the imperfect stage of a *Mycosphaerella* with ascospores measuring 20 to $25 \times 3.5$ to 4.25 μ. This *Mycosphaerella* was found by Roark on overwintered leaves of 2 raspberry species, *Rubus strigosus* Michx. and *R. parviflorus* Nutt., and on 2 blackberry forms, *R. allegheniensis* Porter and *R. hispidus* L., in a restricted section of Wisconsin.

As far as is known by the writers no one in the United States except Roark has demonstrated the relationship between a *Septoria* on *Rubus* and a *Mycosphaerella.*

The very excellent original draft of Roark's doctor's dissertation[2] from which the published extract was taken was examined by the writers. The unpublished paper shows that Roark isolated an ascogenous fungus from overwintered raspberry leaves. Subcultures of a majority of these isolations formed conidia (secondary conidia) in abundance. When these were sprayed on raspberry leaves with an atomizer the well known leaf spot resulted; but, when dewberry leaves were inoculated with the conidia, the result was negative. Inoculations made from an ascogenous fungus on overwintered blackberry leaves made, with one exception, nonsporulating colonies on artificial media. Inoculum from the one sporulating culture was applied to 1 Cuthbert red raspberry and 2 Lucretia dewberry plants. Roark's report shows that he secured on 1 dewberry plant "several spots that look like Septoria spots; but no pycnidia." No infection occurred on the other dewberry plant inoculated, nor on the raspberry plant.

Because of the nonsporulating character of his isolates from perithecia on overwintered blackberry leaves, Roark resorted, for inoculum, to fragments of leaves bearing perithecia and depended upon the forceful expulsion of ascospores to the under surface of leaves. Twenty-five such trials were attempted and 2 were considered successful. In one case leaves of *Rubus hispidus,* with an abundance of mature perithecia, were "arranged so that ascospores would be discharged upon the moistened lower surface of cultivated blackberry leaves." The plant was kept under a bell jar for 4 days. On 4 leaflets groups of brown spots developed and, later, 2 pycnidia. Roark stated: "It is possible but not probable that the lesions were caused

[2] Doctor's dissertation entitled "The *Septoria* leaf-spot disease of *Rubus,*" by E. M. Roark; deposited in the Library of the University of Wisconsin, Madison, Wisconsin.

by pycnospores from pycnidia present along with the perithecia on the pieces of dead leaves." In another case Lucretia dewberry leaves were inoculated in the same manner and from the same source. The inoculated leaves developed "many very small red spots but these red spots never developed any further."

In summarizing the results of inoculations using isolates from pycnospores as the inocula, Roark (15) stated: "None of the strains isolated from the raspberry group were found to infect any member of the blackberry group, and none of the strains from the blackberry were found to infect any type of raspberry."

Zeller (21), who made a study of *Rubus* leaf-spot fungi, and examined some of Roark's material on which the latter based his *Mycosphaerella,* stated that "Some question arises concerning the perfect stage of *Septoria rubi* West. . . . We have never seen in Oregon any *Mycosphaerella* on *Rubus* similar to *M. rubi* Roark."

The present report concerns principally the taxonomic status of the raspberry fungus, and evidence is presented to show that the imperfect stage is identical with *Cylindrosporium rubi* Ell. and Morg., and that the ascogenous stage is a *Sphaerulina*, apparently not heretofore described, and for which the name *Sphaerulina rubi* is proposed. This fungus is believed to be different from *S. intermixta* (Berk. and Br.) reported (20) as occurring in Europe on dead branches of *Rubus* and *Rosa,* and having short ascospores measuring 16 to 18 μ long or about one-half the length of ascospores of the raspberry *Sphaerulina.*

The following is a description of the raspberry leaf-spot fungus: *Sphaerulina rubi* sp. nov.

Stat. conid: *Cylindrosporium rubi* Ell. and Morg. (8). Perithecia usually numerous, scattered or in groups, mostly hypophyllous, at first subepidermal, later erumpent, black, conical, ostiolate-papillate, 88 to 140 μ high and 86 to 120 μ wide. Asci fasciculate, sessile, clavate-cylindrical, curved or straight, 44.8 to 70.0 μ long and 9.6 to 15 μ wide, containing 8 spores; outer wall about 2 μ thick; inner wall membranous. Ascospores hyaline, granular, cylindrical, usually curved, pointed at both ends, slightly more so at apex, normally 4-celled, less frequently 6- to 8-celled; 32.0 to 57.6 μ long and 3.5 to 5.8 μ wide. Paraphyses lacking. Hab. in overwintered leaves of *Rubus strigosus* Michx. in Maryland, Missouri, and North Carolina.

Perithecia plerumque numerosa, conspersa, vel caespitosa, vulgo hypophylla, primum subepidermicalia dein erumpentia, atra, conica, papillato-ostiolata, 88–140 μ alta, 86–120 μ lata; asci fasciculati, sessiles, clavato-cylindrici, curvati vel recti, 44.8–70 μ longi, 9.6–15 μ lati, octospori; tunica exteriori circa 2 μ crassa, interiori membranacea; ascosporae hyalinae, granulosae, cylindricae, plerumque curvatae, utrinque praecipue ad apicem acutae, typice 4-, rarius 6–8-cellulatae, 32–57.6 μ longae, 3.5–5.8 μ latae; paraphyses nulli. Hab. in foliis anni praeteriti *Rubi strigosi* Michx. Maryland, Missouri, et North Carolina.[3]

Pycnidial stage: (*Cylindrosporium rubi* Ell. and Morg. (Emended)). On living leaves, causing circular to angular spots, at first greenish-black, later grayish, usually 1 to 2 mm., sometimes 4 to 6 mm. in diameter; pycnidia, epiphyllus, subepidermal; 58 to 80 μ high and 58 to 121 μ wide; wall thin, 1 to 3 cells thick; pycnospores elongate, obclavate, slightly curved to falcate, pointed at one end, 3- to 9-septate, hyaline, 32 to 86 μ long by 3.0 to 4.8 μ wide in region of greatest thickness. Common in living leaves of red raspberry, *Rubus idaeus* L., and black raspberry, *R. occidentalis* L., east of the Rocky Mountains. Also occurring in canes and overwintered leaves.

[3] Latin diagnosis prepared by Miss Edith K. Cash, Division of Mycology and Disease Survey, U. S. Department of Agriculture.

Type specimen of the fungus has been deposited in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland, under No. 71381.

## PATHOGENICITY OF THE RASPBERRY FUNGUS

Although these studies pertain primarily to the raspberry leaf-spot fungus, parallel studies of the fungi from leaf spots on blackberry and dewberry were made in order to present a more complete picture of the heretofore confused status of the *Septoria* pathogens attacking *Rubus*.

Single pycnospore isolates were made from the leaf-spot fungi on blackberry, dewberry, and raspberry; likewise, monosporic cultures were made from ascospores of the *Sphaerulina* on raspberry.

### Inoculation Experiments

The inoculation work was done on especially grown greenhouse plants. The variety Taylor, the most susceptible one available, was employed for the raspberry studies. The Lucretia dewberry and Lawton blackberry were selected to represent the *Eubatus* section of *Rubus*. The plants, with tops cut below the ground line, were taken from the field in the fall and set in 6- or 8-inch pots. From 1 to 5 disease-free shoots grew from each potted root, and when they had attained a height of 8 to 15 inches, they were considered suitable for inoculating. Each shoot bore leaves of varying age and size, from a closed terminal bud to fully mature leaves. Their size and maturity were recorded on tags attached to each petiole when the several leaves were inoculated. The inoculum, a spore suspension in distilled water with a small amount of Santomerse as a spreader, was applied with an atomizer. The Santomerse proved especially advantageous in case of the raspberries, because of the thick tomentum on the lower leaf surface. It was learned earlier that infection took place principally on the lower leaf surface and that water alone did not readily penetrate the tomentum. Inoculated plants were held for 48 to 72 hours either under bell jars or in a moist chamber equipped with an automatic humidity regulator. Temperature regulation for the inoculated plants during the incubation period was found necessary. The raspberry fungus required a somewhat higher temperature for germination and growth than did the blackberry and dewberry fungi. These temperature requirements were met by holding the inoculated plants during the incubation period in greenhouses maintained at the required temperature levels.

For computing the degree of infection the entire leaf, instead of the individual leaflets, was used as the unit. When the raspberry fungus was inoculated on leaves of its host, heavy infection usually resulted. This fungus at no time ever infected either blackberry or dewberry leaves. A large number of lesions appeared after inoculating blackberry leaves with the blackberry isolate or dewberry leaves with the dewberry form. Cross-inoculation with these 2 latter strains on the 2 hosts concerned resulted in

fewer infections than when the isolates were put on their own hosts. The dewberry isolate was never induced to infect raspberry.

The 7 raspberry plants inoculated with the blackberry fungus (Table 1) were of normal size for the purpose, the foliage ranging from unopened buds to old and mature leaves. The blackberry fungus apparently did not infect such leaves, but did attack very small ones on a few other raspberry plants that were abnormal. These plants, having grown too large for convenient handling, were cut back to the ground line. As a result numerous short, weak shoots with very small leaves developed from the underground parts. The limited number of spots observed on these leaves averaged about ½ mm. in diameter, and had 1 pycnidium in each spot. Size and shape of the pycnospores, growth on artificial media, and inoculation on blackberry leaves proved this fungus to be identical with the one ordinarily inhabiting blackberry leaves, and not the one naturally parasitic on raspberry leaves. This experiment was repeated at another time with similar results.

Since the pycnidia commonly found on raspberry canes contain pycnospores similar to those produced on living raspberry leaves, it was thought essential to determine whether or not a relationship exists. Single-spore cultures were made of the cane fungus, and the resulting conidia were applied to raspberry leaves in the usual manner. A total of over 500 lesions developed on 37 of the 50 inoculated leaves. Raspberry plants inoculated with spores of the leaf pathogen developed pycnidia on the canes when held over winter.

Pathogenicity of the ascogenous stage of *Sphaerulina* from overwintered raspberry leaves was demonstrated by inoculating 132 raspberry leaves with conidia from cultures derived from ascospore isolates. A moderately heavy infection of the leaves was obtained. From the number of leaves inoculated, 121 showed a total of over 18,000 spots within 17 to 24 days. This demonstration is thought to be adequate proof of the genetic relationship of the *Sphaerulina* with the raspberry leaf-spot fungus heretofore generally referred to in literature as *Septoria rubi* West. The conidia from the ascospore isolates failed to infect leaves of blackberry and dewberry inoculated under conditions similar to those under which the raspberry plants were held.

## Age and Surface of Leaves Most Susceptible to Infection by the Raspberry Fungus

Since the leaves on each inoculated plant were tagged, described, and numbered on the date of inoculation, it was possible upon completion of the observation, 3 or 4 weeks later, to determine the approximate age, size, and condition of leaves most susceptible to infection.

The size, or condition, of the leaves was classified as 1, the terminal bud; 2, unfolding buds; 3, small, but expanding leaves; 4, leaves ½ grown; 5, full-size leaves, yet immature, somewhat succulent and greenish-yellow; and 6, leaves considered as mature—that is, fully grown, dark green, with firm texture.

These data, involving 256 leaves and over 37,000 lesions, show that only the young leaves are susceptible to infection. The greatest susceptibility of the leaf seems to be during that period when the leaf area is still increasing, and the tissues are succulent. Very small leaves appear to be less susceptible than those a few days older, but this may be due to the smaller area for collecting spores. After the leaves were fully grown and had developed their normal dark green, they seldom became infected in the inoculating chambers.

In the experiments planned to determine which surface of the leaf is more subject to infection, the inoculum was applied by means of a camel-hair brush. It was shown that infections ordinarily occur on the lower surface of the leaves of raspberry (Table 2). The occasional spots that appear when the inoculum is applied to the upper surface are attributed either to faulty technique or injury of the upper epidermis.

TABLE 2.—*Results of inoculating under and upper surfaces of raspberry leaves with pathogens isolated from this species*

| Source of cultures used for inoculum | Plant, and leaf-surface inoculated | Number of plants inoculated | Number of leaves inoculated | Number of leaves infected | Number of lesions appearing |
|---|---|---|---|---|---|
| Pycnospores from living raspberry leaf | Raspberry | | | | |
| | Under | 4 | 30 | 21 | 5,455 |
| | Upper | 2 | 15 | 0 | 0 |
| *Sphaerulina* ascospores from overwintered raspberry leaf | Raspberry | | | | |
| | Under | 1 | 9 | 9 | 387 |
| | Upper | 1 | 8 | 2 | 5[a] |

[a] These infections appeared near the margin of the leaves and may have been due to faulty technique, or the fungus gained entrance through injuries.

## GERMINATION OF PYCNOSPORES, TEMPERATURE RELATIONS, AND CULTURAL CHARACTERS OF THE RASPBERRY FUNGUS

Pycnospores of *Sphaerulina rubi* germinated readily in tap water. In this medium hyphae grew from the pycnospores both terminally and laterally, but most commonly the germ tubes originated from the ends. Evidences of germination, such as swelling and slight elongation, were manifested soon after the pycnospores were placed in water or on nutrient agar. The segments between the septa showed the greatest enlargement and this accentuated the visibility of the crosswalls.

Although this raspberry fungus grew under artificial conditions through a wide range of temperatures, it made its optimum growth at 27° C. Pycnospores germinated in water at 15° C. and produced hyphae 4 or 5 times their length within 72 hours. During the same interval, when held at 27° C. the hyphae attained a length of 10 to 15 times that of the pycnospores.

Isolations of the fungus, obtained from collections made in different parts of the country, showed considerable variation in size of colonies, color of mycelium, and amount of conidial production when grown on corn-meal

nutrient agar. The growth rate of isolates was comparatively slow, some requiring 4 to 6 weeks to cover the surface of an ordinary slant culture maintained at room temperature, while others never attained a diameter greater than 12 to 15 mm. Aerial hyphae varied in amount from sparse to dense, and in color from mouse-grey to white. Color of the submerged mycelium of different isolates varied from white to olive-black, with the olive-black predominating.

In cultures the fungus never produced pycnidia or other forms of functioning fruiting bodies. However, some isolates formed black, spherical bodies on the surface of the medium which resembled fruiting structures but when crushed between a coverglass and slide yielded what seemed to be an oily substance. On nutrient agars the fungus produced conidia profusely, directly from hyphae. Short sterigmata or conidiophores formed along the hyphae, from which several conidia developed successively (Fig. 1, E). Sometimes hyphae formed rings or hyphal coils and sporulated in a manner similar to that described by Kienholz (11) for *Neofabraea malicorticis* and *Gloeosporium perennans*. Then too, several anastomosing hyphae may form a strand or rope and sporulate abundantly. In older cultures the hyphae at or near the surface became closely septate, cell fusions were frequent, the whole surface became sporogenous, and produced a great quantity of conidia, piled up in mounds or spread out over the surface as a slimy salmon-colored to fawn-colored layer (Fig. 1, F).

A simple method for observing the manner of early conidial formation was to plant either pycnospores or conidia on a sterile coverglass, cover with a drop of clear nutrient agar, and invert over a Van Tieghem cell. Such a culture, incubated at room temperature, would yield conidia in less than a week. These occupied a narrow plane between the agar and the coverglass, and could be readily examined at the desired magnification.

## DISCUSSION

The discovery that a *Sphaerulina* rather than a *Mycosphaerella* is the ascogenous stage of the raspberry leaf-spot fungus, which for many years has been considered to be *Septoria rubi* West., offers an opportunity for speculation as to the probable relationship of this fungus with the forms occurring in dewberry and blackberry.

During these investigations the writers did not find an ascogenous stage in blackberry or dewberry material that could be associated with *Septoria rubi*. The *Sphaerulina*, however, has been plentiful in raspberry leaves each year in Maryland.

The fungus observed on raspberry has not been induced to infect either blackberry or dewberry. Cultures made from blackberry infected the dewberry moderately and the raspberry with difficulty. The fungus isolated from the Lucretia dewberry did not infect the Taylor raspberry, but was moderately pathogenic to the Lawton blackberry. In morphological characters and growth on culture media the raspberry fungus differed from the

forms on blackberry and dewberry, the differences being greater in the case of the Lucretia dewberry form than in the case of the blackberry form. When a particular form infected another host species its morphological and cultural characters were not altered. The variations exhibited by the fungi studied, in the opinion of the writers, indicate that more than one fungus, or else widely divergent strains of the same fungus, are pathogenic for *Rubus* species. Other investigators have observed similar variations. Roark (15) stated that the "blackberry strain would not cross to raspberry nor would raspberry strains infect blackberry." He further stated, in discussing the cultural characteristics of Septoria from *Rubus septoria:* "Strains from the same or different hosts varied considerably . . . some readily forming pycnidia, while others formed only masses of needle-shape secondary conidia." Zeller (21) stated: "Varieties of *R. occidentalis, R. strigosus,* and *R. idaeus* are for the most part extremely susceptible to *S. brevispora* and resistant to *S. rubi,* while blackberry species are rather generally attacked by both." Beach (2) inoculated leaves of the black raspberry (*Rubus occidentalis*) and of a species of blackberry with pycnospores taken from the former, and reported: "In 2 weeks all 30 leaves of *R. occidentalis* were thickly spotted with Septoria, but no trace of infection could be found upon the blackberry." He concluded that the results of his experiment "indicated the existence of biologic forms in *Septoria rubi.*"

It is the conclusion of the writers that the raspberry *Sphaerulina* infects neither the blackberry nor the dewberry under natural conditions, and furthermore, the blackberry and dewberry forms probably never infect the raspberry; but it is possible for the forms on blackberry and dewberry to cross-infect under field conditions.

Until there is more substantial proof of the relationship of the leaf-spot fungi from raspberry, blackberry, and dewberry, it seems advisable at the present time to name the raspberry pathogen *Sphaerulina* ***rubi*** n. sp. and retain the binomial *Septoria rubi* West. (*Mycosphaerella rubi* (West.) Roark) to designate the fungus causing the common leaf spot in the *Eubatus* section of *Rubus.*

## SUMMARY

A disease of the raspberry known as *Septoria* leaf spot is common in the United States east of the Rocky Mountains and does serious damage in the southern half of that region. A similar disease is common on the blackberry and dewberry throughout this country.

The pathogen in all 3 hosts has been known for almost a century as *Septoria rubi* Westendorp, and more recently as *Mycosphaerella rubi* (West.) Roark.

The present investigation shows that the ascogenous stage of the raspberry fungus is a *Sphaerulina,* while no ascogenous stage of the blackberry and dewberry pathogen could be found in overwintered blackberry and dewberry leaves.

In artificial inoculations under greenhouse conditions the raspberry leaf-spot fungus was non-pathogenic for blackberry and dewberry leaves. The isolates from dewberry leaves infected dewberry and blackberry but not raspberry. Isolates from blackberry leaves infected blackberry readily, dewberry moderately, and raspberry with difficulty.

The isolate—whether to be regarded as a species, race, or strain—from each of the 3 hosts differs morphologically and physiologically from the other 2 and retains its morphological identity even if capable of infecting another host.

The differences are greatest between the raspberry and dewberry forms; the blackberry fungus is intermediate between the other 2 forms.

## LITERATURE CITATIONS


1. ALLESCHER, ANDREAS. Die Pilze Deutschlands, Oesterreichs und der Schweiz. In Rab. Krypt. Fl. 1, Abt. 6: 847. 1901.
2. BEACH, W. S. Biologic specialization in the genus *Septoria*. Amer. Jour. Bot. 6: 1–33. 1919.
3. BENNETT, C. W. Michigan raspberry diseases. Mich. Agr. Exp. Stat. Sp. Bull. 178. 1928.
4. BERKELEY, M. J. Notices of North American fungi. Grevillea 3: 1–17. 1874.
5. COLBY, A. S., H. W. ANDERSON, and W. P. FLINT. Bramble fruits. Part II. Ill. Agr. Exp. Stat. Cir. 427. 1935.
6. DAVIS, J. J. Notes on parasitic fungi in Wisconsin. Trans. Wisc. Acad. Sci., Arts and Lett. 21: 282. 1924.
7. DODGE, B. O., and R. B. WILCOX. Diseases of raspberries and blackberries. U. S. Dept. Agr. Farmers' Bull. 1488. 1926.
8. ELLIS, J. B., and B. M. EVERHART. North American species of *Cylindrosporium*. Jour. Mycol. 1: 126–128. 1885.
9. GALLOWAY, B. T. Experiments in the treatment of plant disease. Part III. Jour. Mycol. 7: 12–27. 1891.
10. HESLER, L. R., and H. H. WHETZEL. Manual of fruit diseases. 462 pp. Macmillan Co. (New York). 1917.
11. KIENHOLZ, J. R. Comparative study of the apple anthracnose and perennial canker fungus. Jour. Agr. Res. [U.S.] 59: 635–665. 1939.
12. KICKX, JEAN. Flora cryptogamique des Flandres 1: 453. 1867.
13. KLOTZSCH, J. F. Herbarium Vivum Mycologicum (Ed. 1) Cent. 12, no. 1137. 1847.
14. MARTIN, GEORGE. Enumeration and description of the Septoriae of North America. Jour. Mycol. 3: 85–94. 1887.
15. ROARK, E. W. The Septoria leaf spot of *Rubus*. Phytopath. 11: 328–331. 1921.
16. SACCARDO, P. A. Fungi veneti novi vel critici. Ser. II. Nuov. Giorn. Bot. Ital. 7: 300. 1875.
17. ————. Sylloge fungorum 3: 486. 1884.
18. ————. Sylloge fungorum 3: 580. 1884.
19. WESTENDORP, G. D., and A. C. F. WALLAYS. Herbier cryptogamique belge. Fasc. 1–28. 1841–1859.
20. WINTER, GEORGE. Die Pilze Deutschlands, Oesterreichs und der Schweiz. In Rab. Krypt. Fl. 1, Abt. 2: 400. 1887.
21. ZELLER, S. M. Two Septoria leaf-spot diseases of *Rubus* in the United States. Phytopath. 27: 1000–1005. 1937.
22. ————. *Septoria brevispora* (Sacc.) Zeller renamed. Phytopath. 28: 523. 1938.
23. ZUNDEL, G. L. Some fruit diseases in Pennsylvania. U. S. Plant Dis. Rptr. 26: 329. 1942.

# COMPARATIVE TOXIC EFFECTS OF EXTRACTS FROM MILD AND VIRULENT ISOLATES OF TOMATO-WILT FUSARIUM

FREDERICK L. WELLMAN[1]

(Accepted for publication April 4, 1943)

In the course of studies relating to control of the fusarium (*Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R.) wilt of tomato (*Lycopersicon esculentum* Mill.), work has been published that has dealt with numerous investigations on the action of the pathogen. It has been found that isolates of the fungus differ greatly in pathogenicity (16); that dissimilarity in cultural characters or types are correlated with variations in pathogenicity (15); that this variation is correlated with contrasts in physiology (11); that the virulence of the organism may be increased through selective action, that is, development of variants of exceptional vigor (12, 16); and that the populations of virulent types may multiply and become dominant over mild types under certain environmental conditions, as well as in the presence of tolerant host plants (12). The purpose of the research here reported[2] was to investigate the effects of toxic excretory products of virulent and mild isolates, or "strains," of the tomato-wilt *Fusarium*. A number of workers (2, 4, 5, 6, 7, 17) already have shown that toxic excretory products are formed in cultures of the organism, and a recent report (3) indicates such toxic substances are formed inside the parasitized host cells, as well as in artificial culture. Haymaker (4) compared the relative injurious effects of the toxic substances of a highly pathogenic, with those of a mild, strain of the *Fusarium*, and concluded there were evident differences in effects of the secretions from these two divergent strains. Since my studies dealt with virulent and mild strains of the pathogen similar to those described by Haymaker, it was thought advisable to investigate further any differences in effects of their secretions. No attempt was made to obtain any information on the nature of the substances in secretions from the tomato *Fusarium* that cause the toxic effects.

## MATERIALS AND METHODS

In this investigation it was necessary to use a special technique in connection with demonstrating toxic differences. All cultures were grown on agar prepared in accordance with Wellman's formula (12), and the liquids employed were either of this same composition, except that no agar was added, or of the Tochinai composition (9).

Fusarium cultures were grown as follows: Agar Petri plates (9 cm. diameter, containing 25 cc. of medium) were inoculated in the center with

[1] Senior Agriculturist, Office of Foreign Agricultural Relations, formerly Plant Pathologist, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

[2] I wish to acknowledge the valuable assistance of Wilson Levering Smith, Jr. in the studies reported in this paper.

a disk of inoculum and incubated 3 weeks. From around the edges of these plates were cut "standard inoculum-disks," measuring 6 mm. in diameter. A single disk was dropped in an Erlenmeyer flask of 250 cc. capacity, containing 100 cc. of liquid. The inoculated flasks of liquid were then thoroughly agitated, and were incubated in the dark at near 28° C., without further disturbance.

An experiment consisted of comparative tests of a number of series of cultures, each series of different age. The cultures were filtered through No. 2 Whatman filter paper on a Büchner funnel, using a vacuum of 25 inches of mercury. The mats were discarded, except when weighed, and for every different series from 3 to 7 flasks of filtrates of a given age and of a given strain were combined for testing purposes.

In reporting toxicity results each datum represents the arithmetical mean (standard errors were low, from $\pm$ 0.00 to $\pm$ 0.79, largely around $\pm$ 0.06) of observations on from 5 to 18, usually 8, treated plant tops.

The two isolates of *Fusarium bulbigenum* var. *lycopersici* involved in these studies have been described in other publications (11, 12). They differed markedly in cultural appearance and in pathogenic reaction (11). The one (11) described as R5–6 has here been designated as "V" to indicate its relatively high virulence under the experimental conditions. It caused death of susceptible Bonny Best tomato seedlings within 5 to 7 days after inoculation, and killed tolerant Marglobe seedlings in 20 to 24 days. On Wellman's agar this V strain grew slowly, developing a white, powdery, fine, raised and woolly colony. It produced no macrospores but abundant typical unicellular microspores and chlamydospores (18), and had a sweetish aldehyde odor that became ammoniacal and finally disappeared with age. The other isolate, previously described (11) as A15–8, was designated as "M" to indicate its relatively mild infectiousness. It did not seriously injure Bonny Best seedlings, although it produced some wilting of cotyledonary leaves and occasionally affected one or two leaflets on a basal leaf. It caused no observable wilt of Marglobe. On agar it grew more rapidly than V, and developed a dark colored, slimy colony with a few moist, coarse, twisted and tangled "threads" over its surface. It produced large numbers of typical 4-cell, crescent-shape macrospores, unicellular microspores, and chlamydospores (18), and had a yeasty to sourish odor that became ammoniacal and finally disappeared with age. The Bonny Best and Marglobe strains of tomato used for testing were the same as those employed in previous work (15, 16). Comparative effects on these tomato varieties showed that toxic symptoms, *per se,* did not differ although Bonny Best showed a little greater severity in effect in parallel tests.

Dependable toxicity reactions on host plants were successfully obtained when solutions were tested on excised tops from month-old seedlings grown in a standard manner (13) and selected for uniformity. These tops were cut off in the greenhouse while turgid, dropped in water, and carried to the

laboratory where the cut end of each plant was immersed in 30 cc. of test solution in a test tube of 16 mm. by 150 mm. inside dimensions. It was found that nondiluted filtrates from cultures often were so toxic as to cause very rapid plant collapse. When plant tops wilted or collapsed too quickly it was obviously impossible to secure more critical distinctions between comparative toxic disturbances. Trials showed that a good dilution for testing was 1 part of filtrate to 2 of ordinary tap water. Also, it was found that it was neither necessary to filter cultures aseptically nor to sterilize test solutions by filtration through bacteria-excluding filter candles. Care was exercised by washing procedures to avoid contamination between filtrates for test series. For comparative purposes in each experiment, plain tap water was used as one control and another was made from a dilution of liquid medium that had been inoculated in the standard manner but filtered immediately after agitation with the standard inoculum-disk. Spore suspensions to use as a control series to test for toxic effect were prepared also in several instances by gently washing off spores from the agar surfaces of Petri-plate cultures 3 to 6 weeks old.

It has been demonstrated by many workers (1, 2, 4, 5, 6, 7, 17, 19) that excised tops of tomato seedlings wilt when treated with culture extracts not only from the common tomato-wilt *Fusarium*, but from other fusaria, as well, and indeed from fungi of unrelated genera. These results have been further substantiated, as I have found such wilting caused by *Fusarium bulbigenum* var. *lycopersici* (all of the 5 different types (15) of isolates), *F. bulbigenum* var. *niveum* (E. F. Sm.) Wr., *F. oxysporum* var. *cubense* (E. F. Sm.) Wr. & R., *F. oxysporum* var. *aurantiacum* (Lk.) Wr., *F. oxysporum* Schl. f. 2 Wr. (*F. hyperoxysporum* Wr.), *F. sp.* (causing tomato wilt (14)), *Alternaria solani* (E. and M.) J. and G. (several isolates of different types), *Alternaria tomato* (Cooke) Brinkm., *Alternaria sp.* (small-spored, catenulate type), *Penicillium roqueforti* Thom (Strain #160–18 from Charles Thom), and *Aspergillus niger* van Tieghem. It is thus evident that no specificity for wilting of excised tomato tops can be claimed for *F. bulbigenum* var. *lycopersici*. It has been noted, however, that there are different degrees of injury from toxic liquids from this latter organism and that the variations are correlated with dilution, as well as other factors.

All toxicity tests were made in the laboratory at from 26 to 31° C., and were well protected from sunlight or direct air currents. It seemed that the most conclusive wilting developed in the laboratory when the time selected for testing toxic action occurred during a settled period of dry weather.

Haymaker's (4) indices of toxicity as exhibited on plant tops treated with toxic solutions, were described by him as follows: "stem-drooping = 2; leaf collapse = 6; leaves water-soaked = 8; leaves dry and brittle = 10." After some trials it appeared that under my experimental conditions, a different progression of symptoms occurred. It started with slight darkening around the hydathodes at the apices of serrations on the leaf margins, and

advanced by regular steps through varying necrotic and wilting symptoms on leaves to their shrivelling and desiccation. Stem drooping followed by wilt and collapse was considered as a symptom following occurrence of leaf effects. The progression of symptoms was noted in detail in many experiments and the stages of response were finally listed, arranged in order of severity, and given numerical values (Table 1) that were somewhat com-

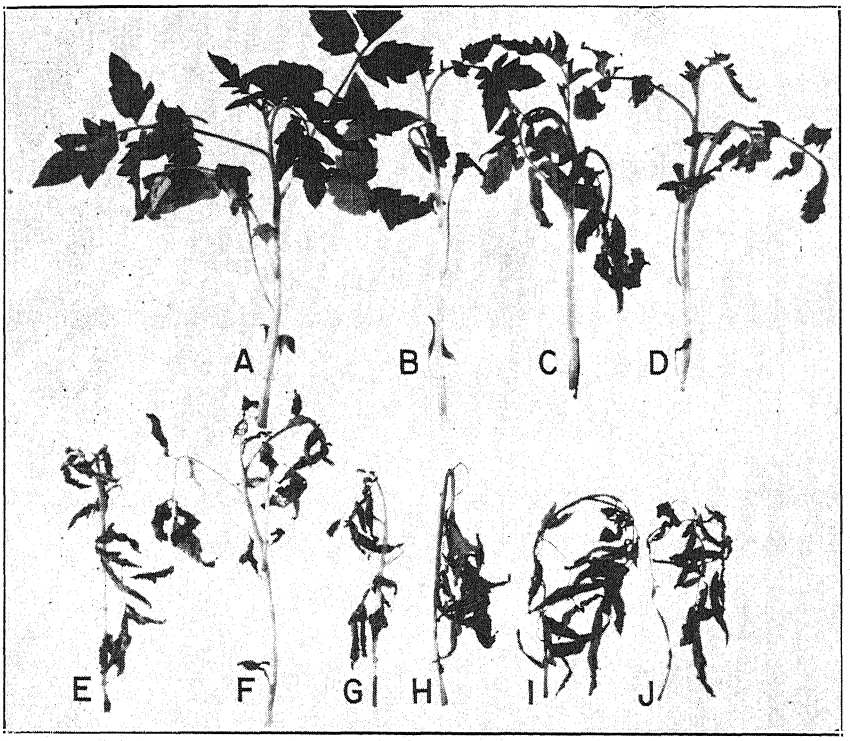



FIG. 1. Different symptoms of toxic effect on excised tops of Bonny Best tomato plants, caused by toxins in filtrates from cultures of tomato-wilt *Fusarium*. A. Included for comparison. No injury, stem immersed in water. B, C, and D. Tests of filtrates taken from mild *Fusarium* isolate during period of its most vigorous growth. E, F, and G. Tests of filtrates taken from virulent isolate during period of its most vigorous growth. H. Test of filtrate from virulent isolate after mat began to show signs of degeneration. I and J. Tests from mild and virulent strains, respectively, during the period in which the mats showed most marked and rapidly changing appearance of degeneration from "staling." Note that E, F, and G appear more seriously injured than B, C, and D. I and J, however, exhibit equally severe collapse. Epinastic effects (see footnote 2, table 1) still observable in C, D, and I. However, when toxic effects were excessively rapid, ending in such severe collapse as in H, I, and J, wilting was seen but epinasty did not occur.

parable to similar values used (9) in estimating pathogenicities of isolates of the tomato-wilt *Fusarium*. The toxic values may be grouped as follows: 0 = no observable injury; 1, 2, 3 = mild injury; 4, 5, 6 = moderately serious; 7, 8, 9 = very serious; 10, 11, 12 = severe; 13, 14 = very severe; and 15 = death.

The most dependable data were from the evaluations taken at 17 to 21 hours after the cut ends of plants were immersed in the test solutions. In

making toxic evaluations, final data were secured by successive recording of evaluations from each experiment at intervals of a few hours during 12 to 48 hours after the tests had been started. At the earliest periods of examination the plants did not appear to exhibit full toxic effects, and after about 26 hours the responses were likely to be irregular and unduly accentuated.

### DIFFERENT TYPES OF TOXIC SYMPTOMS

In these studies there appeared to be two general types of symptoms (Fig. 1) : those confined largely to buds, leaflets, and petioles; and those that

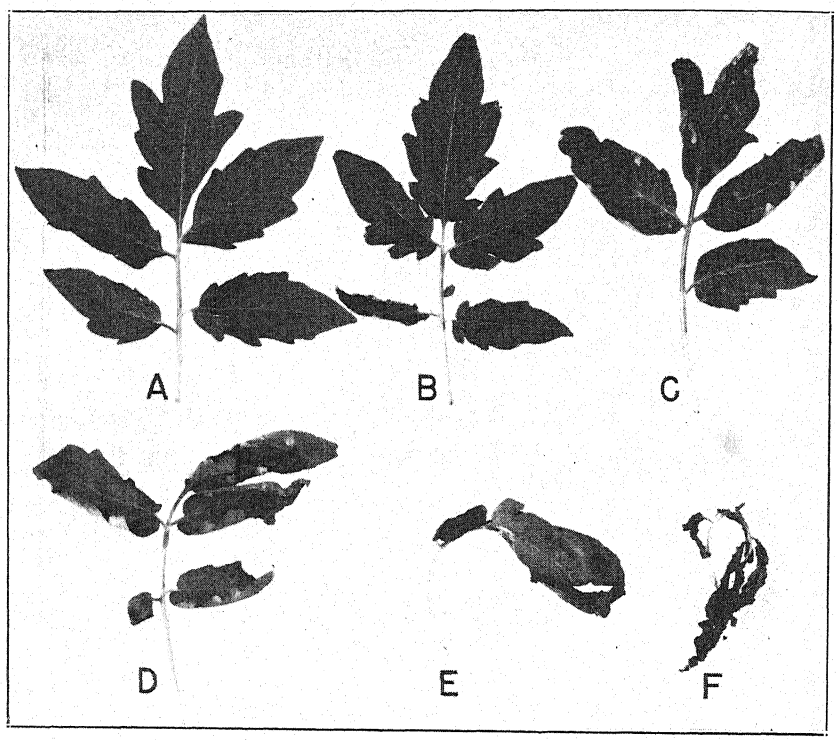



FIG. 2. Symptoms on leaflets of tomato (Bonny Best) of various stages in severity of toxic effect caused by treatment with filtrates of the virulent strain of the tomato-wilt *Fusarium*. For toxic values compare with table 1. Excised plant tops were put in solutions of filtrates from liquid cultures of various ages. A. Included for comparison. No injury, plant top in water, toxic value 0. B. Early symptoms of leaflet injury, plant with toxic value 2. Serrations damaged around the margin of the leaf blades, and one or two small necrotic spots are present in lamina of each leaflet. C. Moderately serious injury, plant with toxic value 4. Increased severity of injury around leaf edges, slight cupping of, and numerous necrotic spots in leaf lamina. D. Serious injury, plant with toxic value 6. Collapse of edges of cupped leaflets, and epinasty. E. Very serious injury, plant with toxic value 8. Wilt of leaflets and epinastic petiole starting to wilt. F. severe injury, plant with toxic value 10. Leaflets crisp-dry and petiole wilted.

consisted of a rather precipitate wilting and collapse of the stem and the whole plant top. These observations were made under the most ideal conditions of experimentation, as outlined above under "Materials and Meth-

TABLE 1.—*Toxic symptoms given numerical values (toxicity indices) and arranged in order of progressive severity of damage. Tests made on excised tops of tomato seedlings*[a] *in solutions from culture filtrates of the tomato-wilt Fusarium*

| Toxicity index | Injury on foliage | | Injury on stem | |
|---|---|---|---|---|
| | Leaf blades | Petioles | Apex and bud | Main stem |
| 0 | No injury | No injury | No injury | No injury |
| 1 | Serrations necrotic on margins of lower leaves | do. | do. | do. |
| 2 | Serrations necrotic on margins of lower and middle leaves, with few small necrotic spots in lamina | do. | do. | do. |
| 3 | Serrations necrotic on margins of all expanded leaves accompanied by slight cupping, many necrotic spots in lamina | First signs of epinasty,[b] small deflection | Slight injury to serrations on bud leaves | do. |
| 4 | Leaves mildly cupped, necrotic spots on laminae, large basal leaflets wilting | Moderate epinastic deflection | Injury more extensive on bud leaves | do. |
| 5 | Extensive areas of collapse on leaves, wilting more extensive | Serious epinasty | Severe injury | do. |
| 6 | Wilting accompanied by severe cupping, edges collapsed | Severe epinasty, some petioles losing turgidity | Injury on bud leaves more extensive, tips flaccid | do. |
| 7 | Severe wilt | First wilt | Tips of bud leaves collapsing | do. |
| 8 | Severe wilt, edges starting to dry | All older petioles wilted | All of bud leaves necrotic | do. |
| 9 | Leaf edges crinkled and dry | Wilting with collapse | Tip bud and leaves crisped | do. |
| 10 | ..... | Collapsed | Tip dead but erect | do. |
| 11 | ..... | ..... | Tip of stem wilted | Slight droop of top |
| 12 | ..... | ..... | Crisp dry | ⅓ of top collapsed |
| 13 | ..... | ..... | ..... | ½ of top collapsed |
| 14 | ..... | ..... | ..... | All wilted |
| 15 | ..... | ..... | ..... | Complete collapse |

[a] Seedlings used were of both Bonny Best and Marglobe varieties, grown according to previously described method (13), 4 to 5 weeks old, about 5-leaf stage, in a warm greenhouse, in 3-inch pots. Tops cut off at ground line below cotyledons, dropped in water and then cut ends immersed in test solutions.

[b] Epinasty, as has already been pointed out (10), is typical of an early wilt symptom, and in these studies was characterized by downward deflection of turgid and unnaturally stiffened petioles, in contradistinction to the droop and flaccidity of ordinary wilting.

ods." It was requisite that the respective ages of the cultures tested be of such spacing and continuity that the progressive changes in toxicity of filtrates could be readily followed.

While there was overlapping in toxic symptoms the stages in change were sufficiently distinct to permit their arrangement in progressive order (Table 1). It was repeatedly noticed that symptoms as indicated for toxic

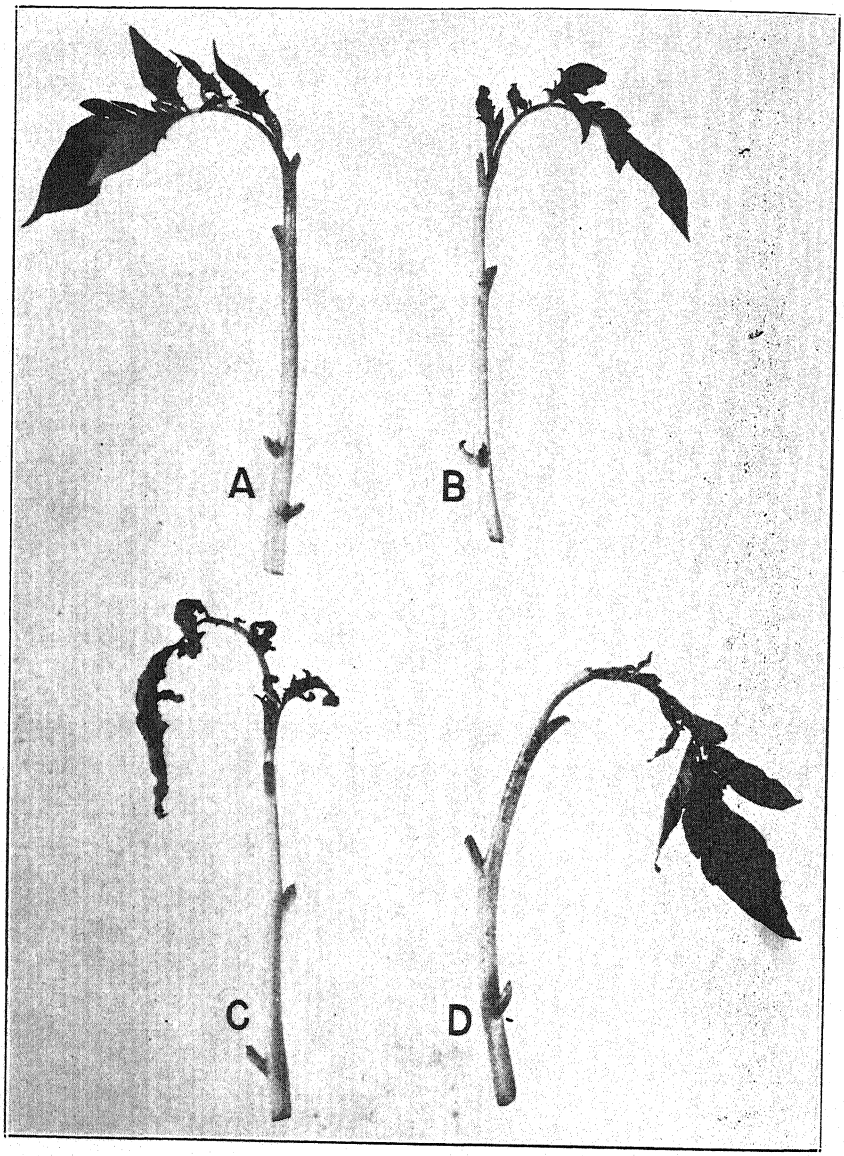



FIG. 3. Symptoms of toxic injury on stem tips and terminal buds of excised Bonny Best tomato plant tops treated with filtrates of growing cultures of wilt *Fusarium* (*cf.* Table 1). A. Included for comparison. No injury, plant in water, toxic value 0. B. Moderately serious injury, effect on edges of youngest tip leaflets, toxic value 5. C. Serious injury, bud and leaflets necrotic, toxic value 9. D. Very serious injury, leaves collapsed, but wilting and stem apex drooping. In another two hours all leaves and bud would have been crisp-dry.

values from 1 to about 10, were developed in filtrates from fresh, well-grown cultures, and symptoms that indicated toxic values from a little above 10 to 15 were from filtrates of cultures that had passed from the stage of vigorous growth to a slightly staled condition. Observations on toxic symptoms

that occurred on plant tops about 18 hours after their cut ends had been immersed in the test filtrates, eliminated somewhat the complications due to excessive drying of toxin-damaged plant parts, that might easily conceal the difference between the collapse from extreme and rapid toxic action that ends in drying, and the slower accumulation of injurious effects that might also end in a desiccated appearance.

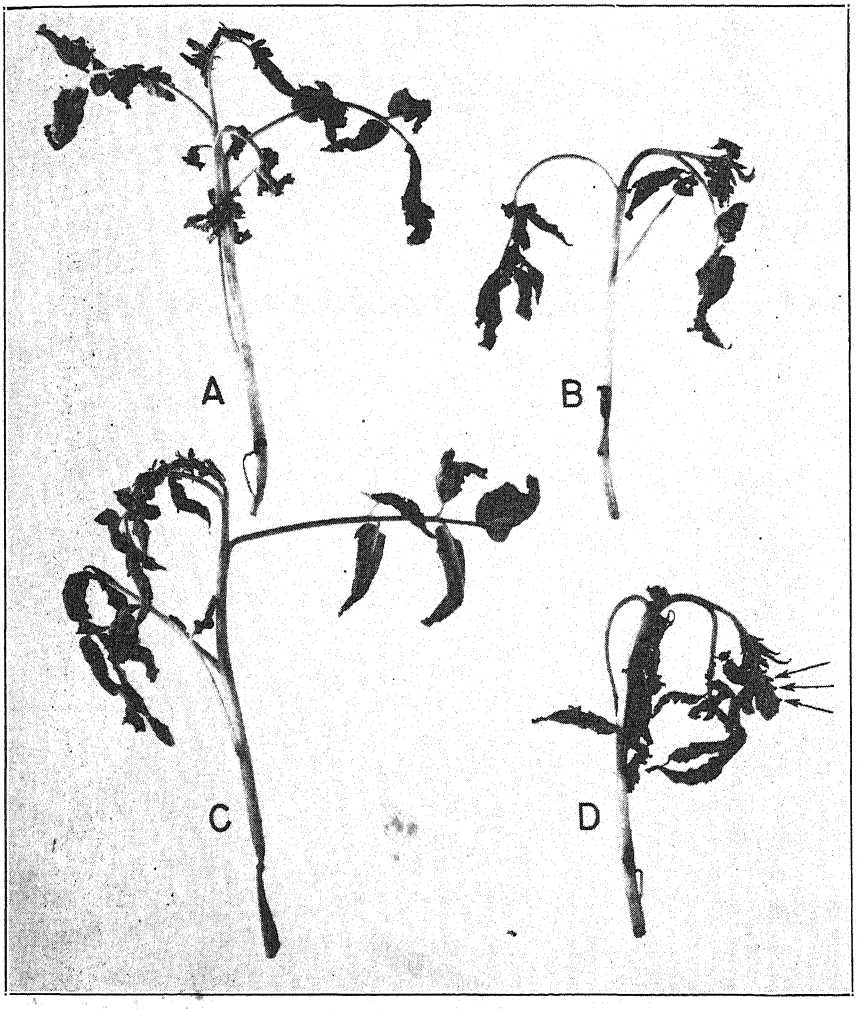



FIG. 4. Comparison of symptoms of different types of toxicity (*cf.* Fig. 1), on excised tips of Marglobe tomato plants, produced by cultures of the wilt *Fusarium*. A and C. Severe injury by filtrate of vigorously growing mats. Shriveled leaflets not quite crisp-dry, epinasty of petioles to be followed shortly by sagging and wilt. B. and D. Wilting response just prior to collapse, due to filtrates from cultures in which mats were showing macroscopic signs of degeneration. Wilting of petioles was not preceded by epinasty. In D, leaflets of the upper leaves were succulent and still showed darkening (indicated by arrows) at hydothodes on serrations around leaf margin. This combination of symptoms occurred rarely in cases of incipient and rapid stem collapse.

It is worth emphasizing that the results of toxic effect from relatively young cultures consisted of symptoms that were almost wholly confined to the leaves. Such symptoms (Fig. 2) were characteristic of plant tests made on filtrates from cultures in which the fungus mats were still in an active state of growth. The symptoms were different in tests from somewhat older cultures. However, it was of interest that, later on, after quite a number of weks of incubation and extreme degeneration of the fungus mats, such leaf symptoms without stem effects were again observable. While these symptoms were of the same general type as those shown in figure 2, they differed from them in that the plants were apt to be a little more flaccid without actually wilting, and the leaves have a slightly grayish cast.

The toxic effects on stems seemed to be of diagnostic value in relation to the condition of the culture from which the filtrates were obtained. Marked stem wilting and collapse as in figures 1, 3, and 4, appeared from treating plants with filtrates from cultures in which the fungus was no longer growing but had recently become "stale" and was in the process of apparent degeneration. In this connection it is well to note that under certain conditions stem collapse occurred so rapidly that leaflets might not be seriously necrosed before wilting (Fig. 4) even though parallel tests with filtrates from younger, vigorously growing cultures might cause extremely severe leaf injury.

### COMPARISONS FROM CULTURE FILTRATES

The object of these studies was to compare differences in toxicity of the filtrates between the V isolate that was extremely pathogenic on tomato plants and the M isolate that was very mild in its effect. Preliminary experiments determined the methods to be employed, and assays were made to record in a numerical manner the varying degrees of toxic injury. A total of 10 experiments were conducted in which tests were made on about 2000 excised plant tops. It is from 8 of these tests that the representative data were secured that are presented in table 2, and from which the graphs were drawn (Fig. 5). Findings from 2 of the experiments were corroborative in nature, but were not included in these data because of the relatively small number of series involved.

As has already been mentioned, it appeared that when a sufficiently planned, extensive experiment was conducted and filtrates were studied from cultures of the proper ages, a regular succession of toxic effects would be observed, as in figure 5 and table 2. The tests of media immediately after inoculation, in which no fusarial growth had occurred, always caused greater toxic effects than were seen on plant tops in tap water or in spore suspensions. The beginning of growth of *Fusarium* actually reduced the toxicity of the liquid in which the spores had germinated and hyphae were in an early stage of development (Fig. 5). This beginning period of mild toxicity lasted for about the first week of incubation in V cultures, and more than twice that long in M cultures. In the V cultures there occurred

during about the 6th to the 12th days of incubation, a considerable increase in toxic effect from the culture filtrates, while in the M cultures this occurred from about the 11th to somewhat past the 30th day.

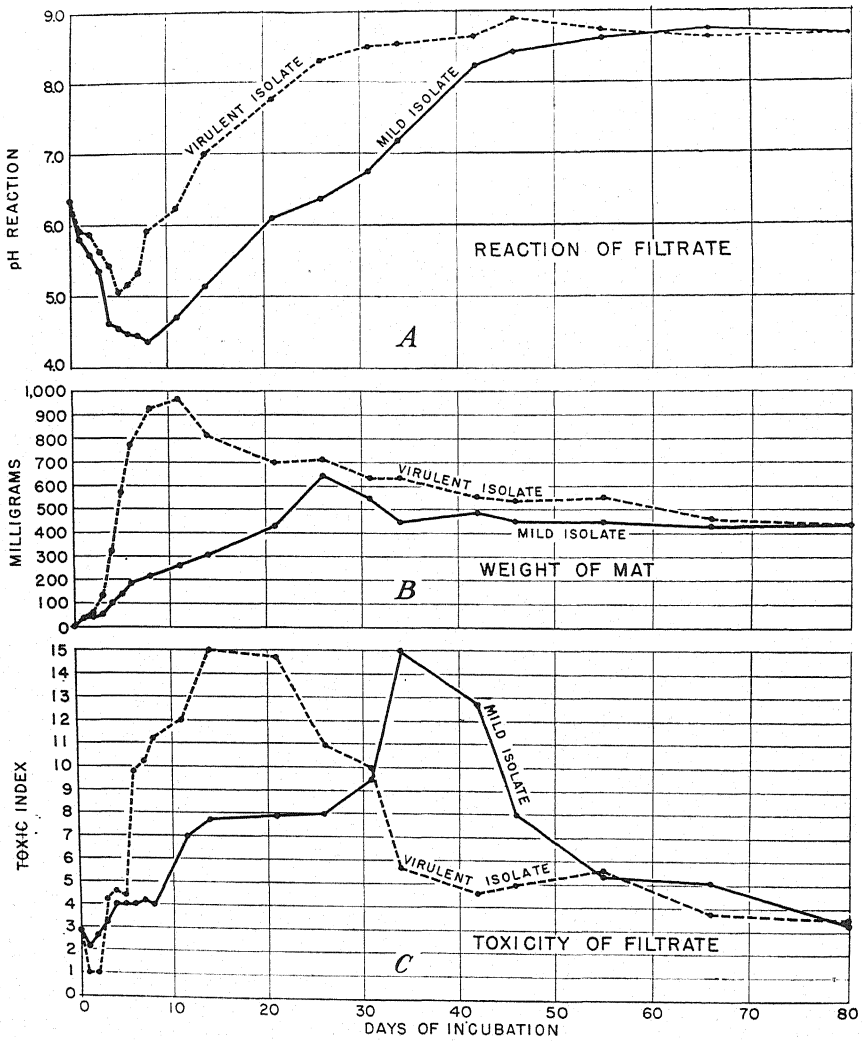



FIG. 5. Results from one typical experiment to compare differences in toxic effect (*cf.* Table 1) of filtrates from cultures at different ages of a virulent and a mild strain of the tomato-wilt *Fusarium*. A. Each pH value is the mean of 3 determinations for each of 3 flasks. B. Each weight is the mean for 3 air-dry fungus mats. C. Each toxicity index is the mean of evaluations of 10 excised Bonny Best tomato plant tops with cut ends immersed in filtrate solutions. Data for A and B secured by methods previously described (11).

It may be seen further (Fig. 5, compare with Table 2) that this period of higher toxicity in both V and M cultures was correlated with the most rapid growth in flasks, as indicated both by increase in mat weight and

TABLE 2.—*Comparison of toxic effects from filtrates of cultures of Fusarium bulbigenum var. lycopersici, from virulent (V) and mild (M) isolates tested at different periods of development*

| Experiment No.[d] | Test plant | Isolate | Toxicity of filtrates when mycelial growth of mats was in condition indicated | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Optimum vigor[a] | | First staling[b] | | Late degeneration[c] | |
| | | | Toxicities[e] | Age of cultures | Toxicities | Age of cultures | Toxicities | Age of cultures |
| | | | | *Days* | | *Days* | | *Days* |
| 1 | BB[f] | V | 10.0–11.0 | 7th to 11th | 15.0–14.8 | 13th to 21st | ..... | ..... |
| | | M | 7.2–8.0 | 13th to 31st | 12.8–10.1 | 35th to 42nd | ..... | ..... |
| 2 | BB | V | 9.2–12.0 | 5th to 9th | 15.0–15.0 | 14th to 26th | 4.7 | 80th |
| | | M | 8.3–8.3 | 14th to 26th | 15.0–12.3 | 34th to 42nd | 4.3 | 80th |
| | Mg[g] | V | 8.7–8.8 | 5th to 9th | 13.7–13.7 | 14th to 21st | 3.2 | 80th |
| | | M | 6.2–6.4 | 14th to 26th | 14.2–12.1 | 34th to 42nd | 3.5 | 80th |
| 4 | BB | V | 10.5–10.0 | 6th to 10th | 13.0–12.0 | 18th to 34th | 2.0 | 95th |
| | | M | 5.2–4.7 | 14th to 31st | 14.7–10.4 | 34th to 55th | 2.4 | 95th |
| | Mg | V | 9.4–8.5 | 6th to 11th | 14.7–9.8 | 21st to 31st | ..... | ..... |
| | | M | 5.0–4.5 | 21st to 31st | 15.0–12.5 | 34th to 55th | ..... | ..... |
| 5 | BB | V | 9.0–8.0 | 5th to 11th | 14.2–14.1 | 14th to 34th | 2.2 | 328th |
| | | M | 4.0–4.0 | 16th to 27th | 11.0–11.0 | 31st to 46th | 2.3 | 328th |
| | Mg | V | ..... | ..... | 10.0–10.0 | 21st to 31st | 1.7 | 328th |
| | | M | ..... | ..... | 9.4–9.0 | 33rd to 46th | 1.4 | 328th |
| 8 | BB | V | 10.4–8.2 | 6th to 10th | 13.0–12.0 | 14th to 21st | 1.6–0.8 | 115th and 330th |
| | | M | 8.8–7.2 | 16th to 30th | 12.1–10.8 | 38th to 50th | 2.7–2.3 | 115th and 330th |
| 9 | BB | V | 5.0–5.0 | 6th to 11th | 12.0–11.1 | 14th to 27th | ..... | ..... |
| | | M | 4.0–3.0 | 18th to 27th | 12.3–7.6 | 34th to 42nd | ..... | ..... |
| | Mg | V | 6.0–6.0 | 8th to 11th | 12.0–8.9 | 14th to 21st | 3.5–2.8 | 100th and 202nd |
| | | M | 4.0–3.8 | 21st to 27th | 9.1–7.0 | 34th to 42nd | 3.5–4.3 | 100th and 202nd |
| 10 | BB | V | 8.0–7.5 | 5th to 12th | 11.3–9.0 | 16th to 20th | 6.0 | 412th |
| | | M | 5.4–4.6 | 16th to 27th | ..... | ..... | 6.0 | 412th |

[a] Mat turgid, floating, adhering firmly to inside walls of flask, surface of liquid solidly filled with growth of cheesy to brittle consistency and of bright colored glistening appearance.

[b] Mats flabby, loosened from attachment to walls of flask, partially submerged, softening, becoming limp, and of dull appearance.

[c] Mats soft, submerged, gelatinous, and of slimy, disorganized appearance.

[d] Data from experiment 3 are presented in graphs shown in figure 5. In experiments 6 and 7, the series were too few and spaced too far apart to give the detailed and proper continuity for the observer to adequately follow the rise and fall in toxicity of successively aged cultures.

[e] Mean values from tests on 5 to 18 plants in each series. Standard errors ranged in most cases from ± 0.0 to ± 0.36. In experiment 6, due to lack of space, tests were confined to 5 plants for each test-series. These plants were also slightly larger than usual and irregular in growth. Errors for tests in experiment 6 were from ± 0.42 to ± 0.78.

[f] BB = Bonny Best tomato variety.

[g] Mg = Marglobe tomato variety.

appearance of fungus growth. Following this (Fig. 5, B) mat weights were in a process of considerable reduction, in V cultures about the 13th to the 28th days, and in M cultures from somewhat past the 30th to after the 45th day. I have pointed out before (11) that, after a certain amount of mat growth, autolytic action results in loss of mat weights. It appears from the present data that it is during this period of degeneration that the liquids, recovered from such autolized cultures, caused extreme toxic responses on excised plant tops. The filtrates producing extreme toxic injury were from cultures about two weeks old for the V isolate, and about a month old for the M. The period of extreme toxicity, once started, lasted about 15 days in both the V and M cultures, and that observation correlates well with the time of most rapid reduction in mat weights. As filtrates aged longer they became much less toxic, so that after a few months to a year or more of standing in flasks, the filtrates were much less injurious and of about equally feeble effect whether from V or M cultures.

## DISCUSSION

The filtrates from vigorously growing cultures of the highly virulent V isolate of the tomato wilt *Fusarium* were notably more toxic than such filtrates from similar vigorous growing cultures of the mild M isolate. It is possible, therefore, that some qualitative difference could be demonstrated between the toxic substances from the M and V isolates. However, the differences might be only in degree of intensity. Furthermore, when filtrates from cultures of either of the isolates were tested shortly after mats in the respective flasks showed signs of autolysis, the liquids were about equally toxic to tomato plant tops. These assays, it is believed, indicate that the toxic principle most likely to be the actual and precise cause of wilt in fungus-infected plants, can more readily be found in *Fusarium* cultures that are still in a vigorous state of growth. After the fungi stop growing in culture flasks and autolysis sets in, the filtrates obtained from such cultures contain a mixture of decay products and secreted toxins.

Observations reported by others corroborate the evidence of differences in toxic effect of the products from old and young cultures. For example, Hikmet Ahmet (5) found that filtrates from 1-week-old cultures of the tomato-wilt *Fusarium* caused the first wilting of tomato plant tops in 24 hours; cultures that were 2 weeks old produced it in 18–22 hours; those 3 weeks old, in 8–10 hours; those 4 or 5 weeks old, in 5–7 hours; and those 6 or 7 weeks old, in 2–3 hours. He observed also that plants treated for 72 hours in liquids from cultures 1 or 2 weeks old bore wilted and flagged leaves but the stems were erect; if treated in filtrates from cultures 3 weeks old the plants drooped, stem and all; and filtrates from cultures 4 to 7 weeks old caused complete stem collapse. Another example is in the work of Haymaker (4), who used cultures that were 5 and 6 weeks old, and considered a lesser period too short a time to produce the most potent toxin content

in culture. From the data he obtained he concluded, "that in the majority of cases excretory products from virulent strains are apparently no more toxic to plants than those from innocuous strains." However, when he used 3-week-old cultures he found that much greater differences in toxic effect were observable between his virulent and mild strains. It would appear that in most of his experiments his results may have been considerably influenced by a preponderance of autolytic products in his test solutions.

It is, moreover, of practical importance that the mild strain of the *Fusarium* appears much slower than the virulent strain in its toxin-production in liquid culture. It has been pointed out above that these differences in progress in toxicity parallel those in growth of the fungus. In previous work I have reported (12) observations indicating that the internal host environment produces a very distinct inhibiting effect on progress of the mild type of the organism in comparison with the virulent. These observations when taken together may give some clue as to the nature of fusarium resistance in the tomato. As advance of the pathogen in the host tissues is more and more impeded by internal environmental conditions occurring in the tolerant or resistant host types, the infected host has a much better chance to overcome the injury from the parasite. It would appear that effective resistance in the tomato plant may be, at least in considerable part, the result of inhibiting or restraining the growth of the organism once it is within the host rather than of neutralization of a toxin.

Two varieties of tomato were used in these studies, Bonny Best, which is markedly susceptible to fusarium wilt under field conditions, and Marglobe, which is highly tolerant. When comparative reactions from toxic effects of culture filtrates were tested on excised plant tops, it was found that differences in injury were distinct in these two varieties but not so great as would be expected from comparative field data on their relative resistance. It is believed, however, that further understanding of the nature of the wilt toxins and the refinement of the techniques of plant assay involved, might result in development of a method for accurate determination of relative resistance of plants to infection by the tomato-wilt *Fusarium*.

## SUMMARY

Studies were made of comparative toxic symptoms appearing on excised tomato plant tops whose cut ends were immersed in filtrates from various aged tomato-wilt *Fusarium* cultures grown in liquid medium. Liquid filtrates from vigorously growing cultures were seriously toxic, causing leaf blade, petiole, and tip bud damage, while filtrates from cultures that had passed the vigorous growth stage and had begun to "stale" caused extreme injury of plant tops including stem collapse. Further aging of liquid cultures appeared to reduce the toxicity of the filtrates to a very marked extent.

Results obtained in comparative assays of a virulent and a mild strain of the *Fusarium* showed that the former strain produced more toxic material

in liquids, and that the filtrates were highly toxic after a much shorter period of incubation than was required by the mild strain. Toxic effects were of about equally extreme severity from staled cultures of either the virulent or the mild strain, and were apparently about equally feeble after long aging of the staled cultures in flasks.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING.
AGRICULTURAL RESEARCH ADMINISTRATION,
UNITED STATES DEPARTMENT OF AGRICULTURE,
BELTSVILLE, MARYLAND.

## LITERATURE CITED

1. ELPIDINA, O. K. On toxins of wilting. Compt. Rend. (Doklady) Acad. Sci. URSS 3: 360–364. 1935.
2. FISHER, P. L. Physiological studies on the pathogenicity of *Fusarium lycopersici* Sacc. for the tomato. Maryland Agr. Exp. Stat. Bull. 374. 1935.
3. GOTTLEIB, D. The presence of a toxin in tomato wilt. Phytopath. 33: 126–135. 1943.
4. HAYMAKER, H. H. Relation of toxic excretory products from two strains of *Fusarium lycopersici* Sacc. to tomato wilt. Jour. Agr. Res. [U.S.] 36: 697–719. 1928.
5. HIKMET AHMET. Untersuchungen über Tracheomykosen. Phytopath. Zeitschr. 6: 49–101. 1933.
6. LUDTKE, M., and HIKMET AHMET. Ueber einen pflanzenlichen Welkstoff. Biochem. Zeitschr. 257: 256–266. 1933.
7. LUZ, G. Über den Stofwechsel von *Fusarium lycopersici* und *Fusarium lini*. Phytopath. Zeitschr. 7: 585–638. 1934.
8. TOCHINAI, Y. Comparative studies on the physiology of *Fusarium lini* and *Colletotrichum lini*. Journ. Coll. Agr. Hokkaido, Imperial Univ. 14: 171–236. 1926.
9. WELLMAN, F. L. A technique for studying host resistance and pathogenicity in tomato Fusarium wilt. Phytopath. 29: 945–956. 1939.
10. ————. Epinasty of tomato, one of the earliest symptoms of Fusarium wilt. Phytopath. 31: 281–283. 1941.
11. ————. Difference in pH relations of some pathogenically variable strains of tomato Fusarium. Phytopath. 32: 271–287. 1942.
12. ————. Increase of pathogenicity in tomato wilt Fusarium. Phytopath. 33: 175–193. 1943.
13. ————. A technique to compare virulence of isolates of *Alternaria solani* on tomato leaflets. Phytopath. 33: 698–706. 1943.
14. ————. A new species of Fusarium causing vascular wilt of tomato. Phytopath. 33: 956–958. 1943.
15. ————, and DOROTHY J. BLAISDELL. Differences in growth characters and pathogenicity of Fusarium wilt isolations tested on three tomato varieties. U. S. Dept. Agr. Tech. Bull. 705. 1940.
16. ————, and ————. Pathogenic and cultural variation among single-spore isolates from strains of the tomato-wilt Fusarium. Phytopath. 31: 103–120. 1941.
17. WHITE, R. P. Studies on tomato wilt caused by *Fusarium lycopersici*. Sacc. Jour. Agr. Res. [U.S.] 34: 197–239. 1927.
18. WOLLENWEBER, H. W., and O. A. REINKING. Die Fusarien, ihre Beschreibung, Schadwirkung und Bekämpfung. (Berlin.) 1935.
19. ZENTMYER, G. A. Toxin formation by *Ceratostomella ulmi*. Science 95: 512–513. 1942.

# DIPLODIA PINEA, THE CAUSE OF A DISEASE OF HARD PINES

ALMA M. WATERMAN[1]

(Accepted for publication March 19, 1943)

## INTRODUCTION

Numerous reports and collections of *Diplodia pinea* (Desm.) Kickx (*Sphaeropsis ellisii*[2] Sacc.) on hard pines have indicated its wide distribution in the United States for some time (16, 29, 30, 31), but it is only within the past decade that it has been recognized as the cause of a disease of sufficient importance to arouse a demand by tree owners and commercial arborists for information regarding the disease and its control. The present paper deals with the taxonomy of the fungus, the hosts and distribution, the pathological effects of the fungus by natural infection of various parts of hard pines and by artificial infection of needles and twigs of the current season's growth. The morphology of *D. pinea* has been discussed by various investigators, particularly Curtis (10) and Birch (4); therefore, it is not included in the present study.

## TAXONOMY OF THE FUNGUS

Desmazières, in 1842, described *Sphaeria pinea* on dead needles of *Pinus sylvestris* L. in France (12, pp. 104–105). In 1867 the fungus was transferred to the genus *Diplodia* by Kickx (17) and was designated as *D. pinea*, with brown one-septate spores measuring 35–40 $\mu \times 16$–18 $\mu$. Since then, a similar fungus on various species of pine throughout the world has been reported as a species of *Phoma, Macrophoma, Sphaeropsis, Diplodia,* or *Botryodiplodia,* according to the collectors' individual interpretations of the material at hand. Hence, the synonymy of the fungus has become considerably confused. Birch (4), in his discussion of *Diplodia pinea* on pines in New Zealand, lists 13 synonyms for the fungus, including species from all of the above genera. Stevens, during a study of certain species of *Sphaeropsis,* made spore measurements from authentic material including type specimens, and various collections of a number of these species from pine specimens filed in the herbarium of the Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agricul-

[1] The writer is indebted to Dr. Neil E. Stevens for permission to examine the slides made from exsiccata and collections of *Diplodia pinea* and to make use of his notes in this study; to Mr. Kenneth F. Aldrich, Assistant to Technician, of the New Haven Office, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, for assistance in maintaining the cultures and making inoculations; and to Mr. Bowen S. Crandall of the Division of Forest Pathology for providing transfers of his cultures.

[2] This species name was published by Saccardo as "ellisi" (24, p. 300) and this spelling has been used previously by the writer (29). Freeman Weiss (32, p. 494) stated: "Published by Saccardo as *S. ellisi,* which may have been a typographical error, as it is spelled *ellisii* in the index. The Recommendations governing orthography in the Rules of Nomenclature prescribe the ii form for epithets derived from specific names ending in a consonant (except -er)."

ture, Beltsville, Maryland. In general his results tend to substantiate the synonymy designated by Birch, as indicated particularly by the size of the spores from the following exsiccata:

*Diplodia pinea* (Desm.) Kickx on dead needles of *Pinus pinea* L. Villa Pamphili, Rome. Mar. 1902. D. Saccardo, Mycotheca Italica No. 955. Spores 27–36 μ × 12–16 μ.

*Diplodia pinea* (Desm.) Kickx on *Pinus radiata* Don. Natal, South Africa. June 1921. Coll. and det. by A. M. Bottomley. No. 14831. Spores 29.7–36.3 μ × 11.8–15 μ.

*Diplodia megalospora* Berk. & Curt. on cones of *Pinus taeda* L. South Carolina. 1874. Berkeley Herbarium No. 5012 (type specimen). Also N. A. F. No. 420. Spores 32–39 μ × 12.8–13.2 μ.

*Diplodia megalospora* Berk. & Curt. on *Pinus nigra* Arnold. Urbana, Illinois. Apr. 7, 1883. Herbarium of A. B. Seymour. Spores 30–36 μ × 12 μ.

*Diplodia megalospora* Berk. & Curt. on leaves of *Pinus halepensis* Mill. Viscosa, Escola, Brazil. Dec. 8, 1929. Coll. by A. S. Mueller. Det. by N. E. Stevens. Spores 35–41 μ × 16–19 μ.

*Sphaeropsis pinastri* Cke. and Ell. on *Pinus sylvestris.* Newfield, New Jersey. 1878. Ellis Herbarium No. 2917. Det. by Cooke. (Type specimen.) Saccardo used this specimen as the basis for establishing the species *S. ellisii* (24, p. 300) since he had already given the name *S. pinastri* (24) to a fungus reported by Léveillé in 1846 as *Phoma pinastri* (20, p. 282). A fungus collected by C. J. Muller at Eastbourne, England, on "fir" cones (presumably Scotch pine) was designated as *Phoma pinastri* Lév. by Cooke in 1878 (7, p. 178). No spore measurements were given in Léveillé's description, but Cooke reported the spores in his material as 10 μ × 6–7 μ. Grove (14) stated that Saccardo used Cooke's measurements in his description of *S. pinastri,* but that the spores in Cooke's specimen are identical in size with those reported for *S. ellisii* Sacc. (*S. pinastri* Cke. and Ell.). He concludes, therefore, that *P. pinastri* Lév. is synonymous with *S. ellisii* Sacc. It is possible that Cooke's specimen labeled *P. pinastri* Lév. may be mixed material, and a portion of it may be identical with *Diplodia pinea* (*S. ellisii*). The inclusion of *P. pinastri* Lév. as a synonym of *D. pinea,* however, cannot be made without a detailed examination of authentic material, not available to the writer. A specimen, numbered 4777 in the Fungi Columbiana and labelled "*Sphaeropsis pinastri* (Lév.) Sacc., on cone bracts of *Pinus nigra* London, Ontario, Canada. April 1915. Det. by J. Dearness," has spores measuring 30–36 μ × 12–13.5 μ and apparently should have been labeled *Sphaeropsis pinastri* Cke. and Ell. or *S. ellisii* Sacc.

*Sphaeropsis ellisii* Sacc. (*S. pinastri* Cke. & Ell.) on fallen cones of *Pinus nigra.* Newfield, New Jersey. Jan. 1894. Coll. J. B. Ellis No. 967. N.A.F. No. 525. Spores 30–36 μ × 12–15 μ.

*Sphaeropsis ellisii* Sacc. on cones of *Pinus sylvestris.* Lombardy. Cavara.—Fungi longobardiae exsiccata No. 95. Spores 30–39 μ × 12–18 μ.

*Sphaeropsis pinicola* Speg. on needles and twigs of *Pinus halepensis*. Fort Cunyngham, Cape of Good Hope, South Africa. Jan. 4, 1913. Coll. and det. by I. B. Pole Evans. Sydow's Fungi Exotici Exsiccati No. 279. Spores 33–39 μ × 14–18 μ.

Additional exsiccata, examined by Stevens, with similar spore measurements, which vary noticeably from those given in the original descriptions of the respective species, are as follows:

*Diplodia conigena* Desm. on cones of *Pinus sylvestris*. Padova, Italy. Jan., 1898. D. Saccardo, Mycotheca Italica No. 355. Spores 28.5–39 μ × 12–15 μ.

*Diplodia conigena* Desm. on cones of *Pinus pinea*. Villa Pamphili, Rome. Mar., 1902. D. Saccardo, Mycotheca Italica No. 956. Spores 30–33 μ × 12–15 μ.

*Diplodia pityophila* Fckl. on dead branches of *Pinus sylvestris*. Budenheim, Austria. Herbier Fuckel 1894. Fungi rhenani No. 538. Herbier Barbey-Boissier No. 2299. Spores 30–36 μ × 12–16 μ.

*Diplodia sapinea* (Fr.) Fckl. on twigs of *Pinus sylvestris*. Padova, Italy. June, 1897. D. Saccardo, Mycotheca Italica No. 160. Spores 28–36 μ × 12–15 μ.

*Diplodia sapinea* (Fr.) Fckl. on twigs of *Pinus sylvestris*. Norfolk, England. Aug., 1874. Coll. by C. P. Plowright. DeThümen, Mycotheca Universalis No. 383. Spores 30–39 μ × 12–13.5 μ.

Birch does not include these species in his synonymy, but Grove gives *Diplodia conigena* as a synonym for his *D. pinastri*, and *D. sapinea* as similar except for spores "sometimes inequilateral" and measuring 20–26 μ × 12 μ. Haddow and Newman (15) found that *Sphaeropsis pinicola* Speg., originally collected from *P. radiata* in Argentina, is also the same as *D. pinea*. These are only a few examples of the confusion encountered in checking over the exsiccata and collections of this fungus. However, Desmazière's *Sphaeria pinea*, changed to *Diplodia pinea* by Kickx, antedates the other species mentioned and therefore *D. pinea* is the preferred designation.

## HOSTS AND DISTRIBUTION

*Sphaeria pinea* was originally described on dead needles of *Pinus sylvestris* in France (12, pp. 104–105). When it was changed to *Diplodia pinea* its habitat was given as needles of *P. sylvestris* and *P. mugo* var. *mughus* (Scop.) Zenari in Belgium, France, and Italy (17). In the United States *D. megalospora* was described on cones of *P. taeda* in South Carolina (3, p. 3) and a similar fungus, *Sphaeropsis pinastri*, was reported on dead twigs of *P. sylvestris* in New Jersey (8, p. 5). Since that time *D. pinea* has been reported under its various synonyms on several species of pine. Many of the early reports did not indicate definitely whether the fungus was found merely on dead parts or on living leaves and twigs, although in some cases it was mentioned as occurring on dead leaves hanging on the trees or on cones. Since the fruiting bodies usually appear only after the leaves or

twigs have been killed by the fungus, most of the exsiccata and collections were from dead material. From the early records, therefore, it is difficult to determine the role of *D. pinea* as a saprophyte or a parasite.

The earliest known reports of *Diplodia pinea* as the cause of a disease of pines were published from the Royal Botanical Gardens, Kew, England, in connection with the occurrence of infected nursery stock and adult trees of *Pinus radiata* and *P. mugo* var. *mughus* at Cape Colony, South Africa (1, 2). In that study inoculation experiments showed that the fungus isolated from *P. radiata* would infect *P. sylvestris* as a wound parasite. A die-back of trees in exotic pine plantations, due to *D. pinea,* has been under investigation in Australia (21, 34). *Pinus radiata* is the most severely attacked, while less susceptible species include *P. ponderosa* Laws., *P. pinaster* Ait., *P. patula* Schl. & Cham., *P. roxburghii* Sarg., *P. caribaea* Mor., and *P. coulteri* Don. In New Zealand two comprehensive studies of the disease on exotic forest pines, particularly *P. radiata* and *P. muricata* Don, were made by Curtis (10) and Birch (4). The latter found that *P. ponderosa, P. nigra poiretiana* (Ant.) Aschers. and Graebn., and *P. contorta* var. *latifolia* S. Wats. also were susceptible.

In Europe various exsiccata and references to collections of *Diplodia pinea* and its synonyms indicate its wide range of distribution, particularly on *Pinus sylvestris,* but its importance as the cause of disease has been mentioned only occasionally. It has been reported as a dangerous parasite on pine in Russia (6), on *P. sylvestris* and *P. nigra* in Rumania (23), and on *P. sylvestris* in Austria (27). Grove (14) stated that *D. pinea* occurs as a serious pest on leaves and young branches of *Pinus* (*sylvestris, mugo* var. *mughus*), "south of England, rather common," and that *D. pinastri* Grove, which seems to be synonymous, is rather common in the British Isles on bark, leaves, and cone scales of *P. sylvestris,* but he made no statement concerning the importance of this latter species as a parasite.

In the United States the first mention of *Diplodia pinea* as a parasite, causing cankers and a dying of the needles of pine in New Jersey, was made by Schwarze (25, pp. 86–87) in 1917. Hedgcock (16) reported *D. pinea* or closely related species from 14 States in the eastern and central United States as a weak parasite often following insect or other injuries on 11 species of both hard and soft pine:—*P. echinata* Mill., *P. griffithii* McClell., *P. flexilis* James, *P. muricata, P. nigra, P. pinea, P. radiata, P. resinosa* Ait., *P. rigida* Mill., *P. strobus,* and *P. sylvestris.* Several of these collections antedate Schwarze's publication and Hedgcock's notes show that the fungus was recognized as the probable cause of disease on *P. nigra* as early as 1908. Reports of the distribution of the disease as indicated by specimens examined by the writer (29, 30, 31) have mentioned its occurrence from 21 States on *P. nigra, P. sylvestris, P. mugo* var. *mughus, P. ponderosa,* and *P. resinosa.* The disease has been reported by Lancaster (18) from Nebraska on *P. nigra* and *P. sylvestris,* and by Slagg and Wright (26) on 10- to 50-year-old trees of *P. nigra, P. ponderosa,* and *P. sylvestris* in Manhattan, Kansas.

Most of these collections have been from ornamental trees, but the fungus has been reported by Crandall (9) on nursery trees of *P. resinosa* causing a collar and root rot and by Slagg and Wright (26) on seedlings of *P. nigra* and *P. ponderosa*, also on 2- and 3-year-old stock of *P. nigra*, *P. ponderosa*, and *P. sylvestris*. These various collections and published reports available to the writer indicate that one or more instances of infection have been found in the following States: Maine, New Hampshire, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Pennsylvania, Maryland, Delaware, Virginia, West Virginia, North Carolina, South Carolina, Kentucky, Tennessee, Ohio, Illinois, Michigan, Wisconsin, Minnesota, Iowa, Missouri, South Dakota, Nebraska, Kansas, and Oklahoma. It is very probable that the disease is even more widely distributed.

Elsewhere in America only occasional collections and reports of *Diplodia pinea* have been found. In Ontario, Canada, Haddow and Newman (15) reported the fungus as the cause of a tip and twig blight of various conifers, in plantations and in the natural forest, but this type of disease was of relatively slight importance. In association with the pine spittlebug, however, the fungus caused serious injury to twigs, branches, and stems of *Pinus sylvestris*, particularly on trees over fifteen years old. In South America *D. pinea* was described under its synonym *Sphaeropsis pinicola* from fallen needles of *P. radiata* in Argentina (15) and as *D. megalospora* on needles of *P. halepensis* in Brazil. The collections of the fungus from the various countries and the literature citations just mentioned indicate that *D. pinea* is the cause of disease of more or less importance in the Western Hemisphere within the range of 30–50 degrees latitude both north and south, and within the same latitude range in the Eastern Hemisphere.

Of the 11 species of hard pine mentioned as hosts for *Diplodia pinea* in the United States, the number and frequency of collections from *Pinus nigra* suggest its high susceptibility (29). *Pinus sylvestris* and *P. mugo* var. *mughus* also are highly susceptible, and occasional instances of infection on *P. ponderosa* and *P. resinosa* have been noted. *Diplodia pinea* is not known to cause any serious disease of well-established trees of these hosts in forest plantations. Only one case of infection has been reported, so far as known, on each on the other 6 species, all of these, except *P. pinea*, being indigenous to the United States. From this it seems evident that it is the exotic pines (*P. nigra*, *P. sylvestris*, *P. mugo* var. *mughus*) that are most susceptible. Boyce (5) points out the fact that certain exotic coniferous forest trees are frequently severely attacked by fungus diseases that are not serious on the same species of trees in their native range. The sparsity of reports on the disease within the native ranges of the susceptible species of pines would seem to indicate that Boyce's statement may well apply to *D. pinea*. This also corresponds with the reports of the disease in Australia (21) and New Zealand (4), where the exotic species *P. radiata* and *P. muricata* are particularly susceptible. Birch (4) considers *D. pinea* as "almost ubiquitous in exotic pine plantations throughout New Zealand, growing as a saprophyte

in dead bark, wood, needles, cones, and general pinaceous débris'' and concludes that it is parasitic ''only on trees weakened as a result of unsuitable growing conditions.'' In the case of ornamental trees of the susceptible species of pine in the United States poor planting methods and unfavorable soil conditions may predispose the trees to infection, but no data on this point are available.

## PATHOLOGICAL EFFECT OF DIPLODIA PINEA

### Natural Infection

The most pronounced symptom of the disease caused by *Diplodia pinea* on hard pines in the United States is the killing back of the current season's growth year after year until the tree becomes stunted and sometimes eventually killed. This usually is a slow process, since most frequently the lowest branches of old well-established trees are first affected, and the disease spreads upward very gradually. If young trees are attacked the killing takes place much more rapidly. Very few instances are known in which large trees have been killed by the disease. This is due to the fact that before the disease has progressed over the entire tree the repeated killing of young terminals has made the tree unsightly and undesirable as an ornamental, and control measures are employed or the tree is removed.

The fungus may attack the current season's growth in any one of a number of ways: by direct infection of young needles as they develop; by infection of the elongating shoot; by the mycelium present in one bud of a terminal cluster spreading to the adjacent buds or into the twig as growth advances; and by mycelium from a twig infected the previous year causing a slow weak growth that rapidly becomes invaded by the fungus. The first of these is observed particularly on newly infected trees. Infection in such cases takes place at the base of the needles and prevents their further growth as the mycelium spreads through the leaf tissue. A few infected needle fascicles may be scattered among the healthy needles of the young shoot. When the needles are brown and dead the fruiting pustules of the fungus appear at the base of the needles and even on the leaf sheaths. The production of the fruiting bodies progresses outward toward the tip of the needle. If the disease is prevalent in the vicinity and seasonal conditions are favorable for spore germination, more extensive infection may occur. On *Pinus sylvestris* the mycelium resulting from infection of the needles may extend from the needles through the tissue of the leaf scars into the twig, forming cankers around the leaf scars. Fruiting bodies soon appear on the twig, and the cankers increase until the twig is girdled. A slight amount of infection is easily overlooked and for this reason the tree owner usually does not realize that his trees are attacked until continued infection follows in successive seasons. Also the fact that the fruiting bodies do not appear until the needles are dead results in uncertainty in making an early diagnosis of the trouble. On *P. mugo* var. *mughus* infection of needles and buds has been found to result in the formation of slowly developing cankers

at the nodes. A die-back of all twigs above the affected nodes occurs very rapidly. Mycelium spreading through the needles into the twigs grows slowly in *P. nigra.* Its effect is not particularly noticeable until it has progressed down the twig toward the node, girdling the twig and thus prohibiting the further growth of the young shoot. Dead needles with sporulating fruiting bodies may hang on these twigs for two seasons and provide a source of inoculum that will infect the new growth.

It sometimes happens that a few needles adjacent to the bud cluster become infected late in the season, and one of these buds, usually a lateral, may become invaded by the fungus. Externally, the only evidence of invasion is an excessive resinosis on and around the bud. Because of the normal resinous condition of buds of *Pinus nigra,* this resinosis usually escapes detection. Sections through such a bud show partial or complete penetration by mycelium. The bud fails to develop during the following season, but the neighboring buds may begin growth normally, only to succumb when the needles are partially grown. Thus, in a cluster of buds, one may be entirely dead, another may develop into a shoot with stunted brown needles, and a third, usually the terminal bud, may produce a fairly normal shoot with a shortened internode, a majority of mature needles, a few stunted diseased needles, and one terminal bud. It is evident that the following year this bud may develop a weak shoot that will soon become infected. This type of infection results in the production of conspicuous clusters of brown, stunted needles with abundant fruiting bodies and an excessive production of resin in both twigs and needles causing a heavy deposit of resin on the bark and the clinging together of affected needles in resinous masses. These symptoms are the most easily recognized by the tree owner and are considered typical of the disease.

Infection of the succulent tissue of an elongating shoot may take place at any time in its development until the needles are about half-grown. The most usual period, however, is that prior to the emergence of the needles from the leaf sheaths. The needles involved in the affected tissue soon cease growth, turn brown, and die. Other adjacent needles may develop to half size but succumb to the effects of the advancing mycelium. Still other needles, particularly near the base of the same shoot, may attain maturity and retain their normal green color apparently unaffected by the fungus.

Although the disease most commonly results in a tip blight or die-back of the young growth, other parts of susceptible pines may become invaded by the fungus. If a cone-bearing twig is infected, the mycelium has been found to penetrate from the twig into the core of the young cones, which then fail to develop. Fruiting bodies of the fungus are frequently abundant on the apophyses of the cone scales of large mature cones or of fallen cones and also on the wings and seed coats. In some cases the trees on which these cones form show no evidence of infection on twigs or leaves, and the fungus seems to be saprophytic. The writer has observed that dead seeds filled with mycelium may be present occasionally, but in most cases the

fungus is confined to the surface of the seed coats. Apparently it infects poorly developed seeds, but is incapable of infecting normal healthy seeds. There is a possibility, however, that its presence on the seed coats might be a source of infection for young seedlings as they emerge from the ground bearing the seed coats on the young leaves. So far as known to the writer this possibility has never been tested. Slagg and Wright (26) reported the infection of pine seedlings 5 months after the seed was sown, but the source of infection was not determined. Birch (4) reports that tests with seed of *Pinus nigra poiretiana* from Canterbury, New Zealand, and *P. ponderosa* from the northwestern United States showed a very small percentage of infected seed. He believed that the fungus occurs as a saprophyte on the surface of the seed coats and in the interior of dead seed.

A collar and root rot of nursery trees caused by *Diplodia pinea* has been reported by Crandall (9) on 3- to 5-year-old seedlings of *Pinus resinosa* in Maryland. Stem infection by *D. pinea* in 7- to 15-year-old trees of *P. ponderosa* and *P. radiata* in stands in New Zealand (4) caused the death of trees if penetration by the fungus occurred near the ground level. A sap stain in southern pine lumber in the United States was attributed by Davidson (11) to *D. pinea* (*D. megalospora*), and a similar sap stain was described by Verrall (28), as caused by an undescribed species of *Diplodia,* possibly a strain of *D. megalospora.* Staining of the sapwood of *P. pinea* from which *D. pinea* was isolated has been studied in detail by Goidanich (13) in Italy in timber and in living trees weakened by severe root pruning or by borers. In New Zealand also (4) the fungus has been found to produce a staining of timber from *P. contorta* var. *latifolia, P. radiata, P. ponderosa,* and *P. muricata,* but living trees are not susceptible "unless in advanced stages of disability."

## Artificial Infection

In a study of tip blight and die-back of *Pinus nigra, P. mugo* var. *mughus,* and *P. resinosa* caused by *Diplodia pinea,* White (33) successfully inoculated the young growing tips of potted trees of *P. mugo* var. *mughus* and *P. sylvestris* in the greenhouse with spores isolated from infected needles of *P. nigra.* Later, Pirone (22) found by inoculation that 3- to 6-year-old trees of *P. mugo* var. *mughus, P. sylvestris,* and *P. nigra* were susceptible to stem infection by *D. pinea,* similar to that described by Crandall (9) from inoculations of 3-year-old seedlings of *P. resinosa.* Haddow and Newman (15) reported the successful inoculation of healthy shoots of *P. sylvestris* in the field with a suspension of spores of *D. pinea* from infected pine twigs of the same host. The inoculum was injected an inch or two below the terminal buds of leading and lateral shoots. Of the 30 shoots inoculated, all showed infection after 3 months.

In the present study the effect of *Diplodia pinea* on the current season's growth of 4 species of hard pine and 1 of soft pine was tested by inoculating young nursery trees of various ages (approximately 5–10 years) in an experimental plot, growing under exceptionally favorable conditions. The

species of pine were as follows:—*Pinus nigra* (trees 2′–4′ in height); *P. sylvestris* (2′–6′); *P. resinosa* (2′–4′); *P. ponderosa* (2′–3′); and *P. strobus* (1½′–3′). Single-spore cultures of *D. pinea* isolated from needles of *P. nigra* collected at Hamilton, Mass., Southold, Long Island, N. Y., and Philadelphia, Pa., and grown on Leonian's synthetic medium (19), were the source of inocula. In a few cases a loopful of freshly exuding spores from pycnidia in culture was used as inoculum, but in most cases immature fruiting bodies or bits of mycelium were substituted for the spores, since the fungus did not fruit readily in culture. The kind of inoculum did not seem to influence the results. Inoculations were made in April, and June, 1934, May, 1935, May, 1936, and June, and July, 1937. Because of the fact that the current season's growth did not develop uniformly on all the trees a limited amount of growth was usually available at any one time when inoculations could be made. Therefore, in some cases, only one inoculation of a kind could be made on each host. The results are necessarily inconclusive but give a general indication of the conditions under which infection may take place. Whenever two or more inoculations were made on one tree, particular care was taken so that these should be on widely separated bud clusters or shoots to prevent the possibility of cross infection. The inoculum was applied to the trees in five different ways: 1. On the surface or at the base of uninjured buds early in the spring before new growth started; 1. On buds injured by piercing the bud scales near the base of the bud with a sterilized needle; 3. On the surface of uninjured leaves of the new growth, near the bases of the leaves when they were about 1 inch long; 4. On leaf scars from which the leaves of the new growth had just been removed; 5. In wounds on young twigs, about 1″–3″ above the node, made by cutting with a sterilized scalpel a small triangular section of bark under which the inoculum was inserted. In all cases after the inoculum was applied the portion inoculated was wrapped with moist cotton, over which was firmly tied a strip of heavy waxed paper. The coverings were removed 1 to 2 weeks after inoculation, depending upon the weather conditions and the rate of development of the current season's growth. Controls were made to compare with the different types of inoculations except that no inoculum was introduced.

The results, as shown by table 1, indicate that uninjured buds of *Pinus sylvestris, P. resinosa,* and *P. ponderosa* may become infected. In 3 cases the new growth from these buds developed until the first needles attained a length of about 1 inch. Both leaves and twigs of the new growth remained chlorotic and stunted, soon turning brown. Early in the following spring the fungus was reisolated from two of the brown twigs. The other infected twig, showing fruiting bodies the next spring, was left on the tree to determine whether the fungus would spread farther. In the fall of the same year, however, there was no indication that the fungus had advanced into the previous year's growth. The twig was removed and the fungus was reisolated from the affected tissue. Two of the inoculations made on uninjured buds resulted in the infection of scattered needles, particularly at the

tip near the terminal bud. These were left on the trees until the following spring, when the advance of the fungus into the twigs became noticeable. The one terminal bud on each of these twigs developed only a weak growth and both needles and twigs of this growth became infected, the twigs being killed back to the nodes. In the fall they were collected and the fungus was reisolated. In another case of needle infection only a few scattered needles became infected the first season. These were left on the tree and slight infection of needles appeared in each of the two following seasons. After the second season the fungus was reisolated from infected needles. In the two remaining inoculations reported from uninjured buds, both of

TABLE 1.—*Results of inoculations of species of Pinus with Diplodia pinea isolated from Pinus nigra*

| Part inoculated | Species inoculated | Number of | |
|---|---|---|---|
| | | Inoculations | Infections |
| Buds uninjured | *nigra* | 1 | 0 |
| | *sylvestris* | 11 | 3 |
| | *resinosa* | 9 | 4 |
| | *ponderosa* | 2 | 1 |
| Bud scales injured by needle puncture | *nigra* | 1 | 1 |
| | *sylvestris* | 6 | 3 |
| | *ponderosa* | 1 | 1 |
| Leaves uninjured | *nigra* | 2 | 1 |
| | *sylvestris* | 3 | 3 |
| | *resinosa* | 3 | 2 |
| | *ponderosa* | 1 | 1 |
| | *strobus* | 1 | 0 |
| Leaf scars—leaves removed | *nigra* | 2 | 1 |
| | *sylvestris* | 1 | 1 |
| | *resinosa* | 1 | 1 |
| | *strobus* | 1 | 0 |
| Twig wounded by slitting bark | *nigra* | 3 | 3 |
| | *sylvestris* | 3 | 3 |
| | *resinosa* | 1 | 1 |
| | *strobus* | 1 | 0 |

which were made on leaders, one lateral bud in each inoculated cluster failed to develop, but the terminal bud put out normal healthy growth. Infection in the lateral bud spread into the main twig, and in both cases cankers developed during the following season. These resulted in a girdling of the twig, killing the growth that had been produced by the terminal buds following inoculation. Fruiting bodies of the fungus appeared on the cankers and the organism was reisolated.

When the buds were injured by piercing with a sterilized needle, infection took place on *Pinus nigra, P. sylvestris,* and *P. ponderosa.* Two instances of infected shoots, one of infected scattered needles, and two of infected lateral buds developing into cankers resulted in symptoms similar to those produced from the infected uninjured buds. On *P. nigra* the inoculated lateral bud on the leader became infected and a canker was gradually formed, beyond which the uninfected terminal buds put out new

growth for three seasons. The following season the canker had developed sufficiently to girdle the stem (Fig. 1, A) and the entire top died back. The fungus was reisolated from the canker, as well as from the other four cases of infection from injured buds reported in table 1. In this series of inoculations the controls produced a fairly normal growth, at first slightly

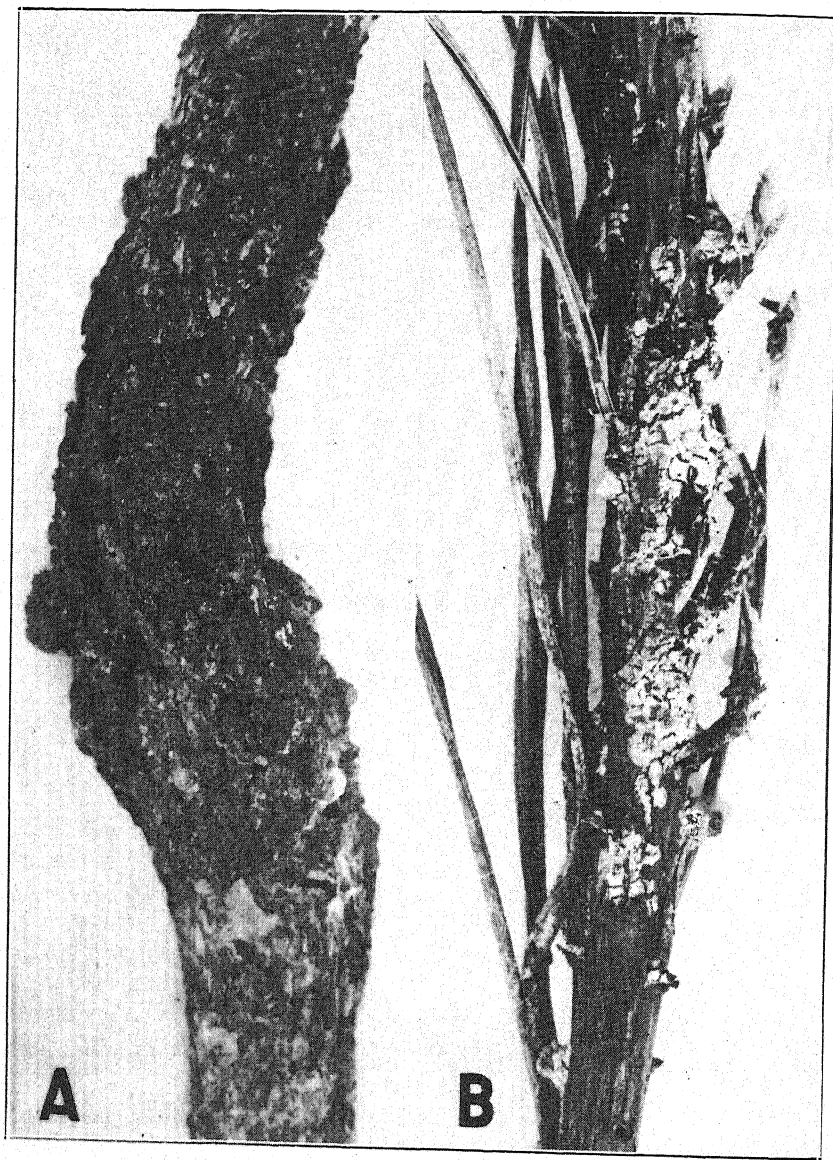



FIG. 1. Cankers resulting from inoculations with *Diplodia pinea*. A. Four-year-old canker from infection of injured bud of *Pinus nigra*. (Approximate × 1½.) B. Canker 3 months after inoculation of twig wound on *P. sylvestris*. (Approximate × 1½.) Photographs by H. G. Eno.

chlorotic, but apparently the piercing of the bud scales with the needle did not permanently injure the growing tissue.

Positive results were obtained from seven of the inoculations on uninjured leaves. Numerous brown needles with fruiting bodies of the fungus were evident in the autumn following inoculation, and small cankers with fruiting bodies were formed on the twigs around the base of the infected needles. Specimens were collected and the fungus was reisolated. The controls in this series were chlorotic at first, soon becoming normal. When the inoculum was applied directly to the leaf scars from which the leaves had just been removed, infection occurred in 3 instances, causing small cankers and diseased needles. The fungus was reisolated from two of the cankers in the autumn following inoculation and from the third canker the next spring. There was no evidence of injury in the controls.

The fifth series of inoculations indicates that the fungus will readily infect the host through wounds. Definite cankers, with considerable resinosis on the infected needles and twigs (Fig. 1, B), as well as an abundance of fruiting bodies on the cankers and surrounding needles, were conspicuous three months after inoculation. A month later, at the end of the growing season, the fungus was reisolated from 5 of the cankers. The other 2 cankered twigs were left on the trees during two growing seasons and the fungus was reisolated from them in the following fall. All the controls in this series showed normal wound healing.

In June, 1934, mycelium from a culture of *Diplodia pinea,* isolated by Crandall from the stem of a 3-year-old seedling of *Pinus resinosa* showing collar and root rot (9), was used as inoculum in four inoculations as follows: two inoculations on uninjured buds of *P. nigra,* one on an uninjured bud of *P. resinosa,* and one on a lateral bud of the terminal cluster on *P. nigra* injured by piercing with a sterilized needle. Positive results were obtained from the third and fourth inoculations. From the uninjured infected bud the new growth developed only partially, with leaves and twig soon turning brown. In the following spring fruiting bodies were abundant on the twig that was killed back to the node, but the fungus spread no farther. In the fall the fungus was reisolated from the diseased tissue. In the fourth inoculation, infection of the injured lateral bud resulted in the formation of a canker at the node. The fungus did not spread beyond that area, however, and was reisolated in the spring of 1938.

## SUMMARY

The disease of hard pines caused by *Diplodia pinea* (Desm.) Kickx (*Sphaeropsis ellisii* Sacc.) is widely distributed in the United States and has also been reported from various countries in both Hemispheres within the latitude range of 30 to 50 degrees both north and south. In the United States the exotic hard pines, *Pinus nigra, P. sylvestris,* and *P. mugo* var. *mughus,* are the most susceptible, but the fungus occurs occasionally on eight other species of hard pine. The disease has not yet been known to cause any

extensive injury in forest plantations but frequently it is of considerable importance on ornamental trees.

The killing back of the current season's growth of ornamental trees for several successive seasons results in the weakening of the trees and sometimes in the death of entire trees. The disease may attack the new growth in any one of a number of ways: by direct infection of young needles; by infection of the elongating shoot; by infection of one bud of a terminal cluster from which the mycelium spreads into adjacent buds or into the twig; and by spread of mycelium from a twig infected the previous year.

Inoculations were made in an experimental plot on young trees of *Pinus nigra, P. sylvestris, P. resinosa, P. ponderosa,* and *P. strobus* using as inoculum mycelium or immature fruiting bodies produced in single-spore cultures of *Diplodia pinea* isolated from infected needles of *P. nigra,* and from stem cankers on *P. resinosa.* Positive results were obtained from the inoculations of uninjured buds of *Pinus sylvestris, P. resinosa,* and *P. ponderosa;* of injured buds of *P. nigra, P. sylvestris,* and *P. ponderosa;* of uninjured leaves of *P. nigra, P. sylvestris, P. resinosa,* and *P. ponderosa;* of leaf scars of *P. nigra, P. sylvestris,* and *P. resinosa;* of twig wounds of *P. nigra, P. sylvestris,* and *P. resinosa.*

The results of the inoculations indicate that *Diplodia pinea* will infect healthy actively growing tissue of buds and leaves but will infect more readily through wounds.

DIVISION OF FOREST PATHOLOGY, BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING, AGRICULTURAL RESEARCH ADMINISTRATION,
U. S. DEPARTMENT OF AGRICULTURE, IN COOPERATION WITH
OSBORN BOTANICAL LABORATORY,
YALE UNIVERSITY, NEW HAVEN, CONN.

## LITERATURE CITED

1. Anon. A pine disease (*Diplodia pinea*). Jour. Bd. Agr. (Great Britain) 14: 164–166. 1907–1908.
2. BANCROFT, K. A pine disease (*Diplodia pinea* Kickx). Roy. Bot. Gard. Kew Bull. Misc. Inform. 1911 (1): 60–62.
3. BERKELEY, M. J. Notices of North American fungi. Grevillea 3: 1–17. 1874.
4. BIRCH, T. T. C. *Diplodia pinea* in New Zealand. New Zealand State Forest Service Bull. 8. 32 pp. illus. 1936.
5. BOYCE, J. S. Exotic trees and disease. Jour. For. 39: 907–913. 1941.
6. CHERNETSKAJA, Z. S. Gribok *Diplodia pinea* (Desm.) Kickx kak opasnyi parazit sosny. Mater. Mikol. i Fitopat. 5(2): 24–28. 1926.
7. COOKE, M. C. British fungi. Grevillea 3: 177–186. 1874–1875.
8. ———, and J. B. ELLIS. New Jersey fungi. Grevillea 7: 4–10. 1878–1879.
9. CRANDALL, B. S. A root and collar disease of pine seedlings caused by *Sphaeropsis ellisii.* Phytopath. 28: 227–229. 1938.
10. CURTIS, K. M. A die-back of *Pinus radiata* and *P. muricata* caused by the fungus *Botryodiplodia pinea* (Desm.) Petr. New Zealand Inst. Trans. and Proc. 56: 52–57. 1926.
11. DAVIDSON, R. W. Fungi causing stains in logs and lumber in the Southern States, including five new species. Jour. Agr. Res. [U.S.] 50: 789–807. 1935.
12. DESMAZIÈRES, J. B. Neuvième notice sur quelques plantes cryptogames, la plupart inédites, récemment découvertes en France, et qui vont paraître en nature dans la

collection publiée par l'auteur. Annales des sciences naturelles II, 17: 91–118. 1842.
13. GOIDANICH, G. Coloration du bois de pin produite par une variété de *Sphaeropsis ellisii* Sacc. Boll. Sez. Ital. Soc. Intern. Microbiol. 7: 181–184. 1935. (Translation by E. H. Ozmun in New Haven Branch Library, Division of Forest Pathology, U.S.D.A.)
14. GROVE, W. B. British stem- and leaf-fungi (Coelmycetes). Vol. II, 406 pp. 1937. Cambridge University Press.
15. HADDOW, W. R., and F. S. NEWMAN. A disease of the Scots pine (*Pinus sylvestris* L.) caused by *Diplodia pinea* Kickx associated with pine spittle-bug (*Aphrophora parallela* Say.). Trans. Royal Canadian Inst. 24(1): 1–18. 1942.
16. HEDGCOCK, G. G. Notes on the distribution of the fungi associated with diseases of conifers. Pl. Dis. Rptr. [U.S.] 16: 28–42. 1932.
17. KICKX, J. Flora cryptogamique de Flandres 1: 397. 1867.
18. LANCASTER, F. R. Pine tip die-back in Nebraska. Pl. Dis. Rptr. [U.S.] 23: 126. 1939.
19. LEONIAN, L. H. A study of factors promoting pycnidium-formation in some Sphaeropsidales. Amer. Jour. Bot. 11: 19–50. 1924.
20. LÉVEILLÉ, J. H. Descriptions des champignons de l'herbier du muséum de Paris. Annales des sciences naturelles III, 5: 249–304. 1846.
21. LUDBROOK, W. V., and N. H. WHITE. Observations and experiments on Diplodia die-back of pines at Canberra, A.C.T. Jour. Counc. Sci. Industr. Res. Australia 13(3): 191–194. 1940.
22. [PIRONE, P. P.] *Sphaeropsis ellisii* on conifers. New Jersey Agr. Exp. Stat. Nursery Disease Notes 10: 43–46. 1938.
23. PRODAN, I. *Diplodia pinea* (Desm.) Kickx in Rumanien. Bulet. Grad. Bot. Univ. Cluj 14: 240–243. 1934.
24. SACCARDO, P. A. Sylloge fungorum. Vol. 3. 860 pp. 1884.
25. SCHWARZE, C. A. The parasitic fungi of New Jersey. N. J. Agr. Exp. Stat. Bull. 313. 226 pp. 1917.
26. SLAGG, C. M., and E. WRIGHT. Diplodia epidemic in conifer seedbeds. (Abstract) Phytopath. 32: 16. 1942.
27. TOBISCH, J. Beiträge zur Kenntnis der Pilzflora von Kärnten. V. öst. Bot. Zeit. 87(4): 273–315. 1938.
28. VERRALL, A. F. Relative importance and seasonal prevalence of wood-staining fungi in the Southern States. Phytopath. 29: 1031–1051. 1939.
29. WATERMAN, A. M. The disease of pines caused by *Sphaeropsis ellisii*. Pl. Dis. Rptr. [U.S.] 23: 93–95. 1939.
30. ————. Diseases of shade and ornamental trees: summary of specimens received in 1939 at the New Haven Office, Division of Forest Pathology. Pl. Dis. Rptr. [U.S.] 24: 157–159. 1940.
31. ————. Diseases of shade and ornamental trees: annotated list of specimens received in 1940 at the New Haven Office, Division of Forest Pathology. Pl. Dis. Rptr. [U.S.] 25: 181–186. 1941.
32. WEISS, FREEMAN. Check list revision. Pl. Dis. Rptr. [U.S.] 25: 494–503. 1941.
33. [WHITE, R. P.] Tip blight of conifers. New Jersey Agr. Exp. Stat. Nursery Disease Notes 9: 38–41. 1937.
34. YOUNG, H. E. The species of *Diplodia* affecting forest trees in Queensland. Queensland Agr. Jour. 46(3): 310–327. 1936.

# STUDIES ON THE MORPHOLOGY, PHYSIOLOGY, SEROLOGY, LONGEVITY, AND PATHOGENICITY OF CORYNEBACTERIUM SEPEDONICUM

S. F. Snieszko[1] and Reiner Bonde[2]

(Accepted for publication, February 11, 1943)

## INTRODUCTION

During the study of ring rot of the potato in the United States and elsewhere during the last few years, a number of interesting and heretofore unreported properties of the causative organism *Corynebacterium sepedonicum* have been described. The main contributions to the knowledge of the nature of the disease and of the biology, morphology, cultivation, and systematic position of the ring-rot organism have been reported by Bonde (1, 2, 3), Dykstra (7), Eddins (9, 10), Racicot *et al.* (15), Savile and Racicot (16), Skaptason and Burkholder (17), and Stapp (20).

However, certain improvements still could have been made in the cultural methods and in the methods of determining the physiological properties of the organism. More information also has been needed regarding the serological properties, the viability and the virulence of this bacterium.

## MEDIA AND CULTIVATION

In or on any of the media recommended for the cultivation and isolation of *Corynebacterium sepedonicum,* this organism grows relatively slowly. At the optimum temperature (22–25° C.) colonies clearly visible to the unaided eye appear in 8 to 12 days. This is a rather long time, and a shortening of the incubation period needed for the isolation or growth of *C. sepedonicum* seemed very desirable. After many old and new media were tried one was found that was particularly satisfactory. The composition of this medium ("4–d") was as follows:

| | |
|---|---|
| Bacto peptone | 3 g. |
| " tryptose | 3 " |
| " yeast extract | 3 " |
| Dextrose | 3 " |
| Distilled water | 1 liter |
| pH 7.0 | |

It was found later that maltose, and to a certain degree lactose, also supported the growth of *C. sepedonicum,* without any appreciable lowering of the pH of the medium during incubation which may accelerate the death of the bacteria. Therefore dextrose has frequently been replaced with maltose (medium "4–m"). In the routine work "bacto technical maltose," as less expensive, was used in place of maltose C.P. For prolonged cultivation, as

[1] Assistant Plant Pathologist, Maine Agricultural Experiment Station, formerly Professor of Bacteriology of the Jagellonian University, Krakow, Poland.
[2] Associate Plant Pathologist, Maine Agricultural Experiment Station.

in stock cultures, one third of the maltose has been replaced by lactose (medium "4–m–l"), inasmuch as lactose also was found to be utilized slowly by *C. sepedonicum* with very slow and moderate lowering of the pH of the medium, even after several months of cultivation. Growth on medium "4" (without any available source of carbon also is relatively fast, but scant in volume.

Experience has shown that stock cultures, maintained at room temperature, survive much longer in a liquid medium than on a solid one. Agar slant cultures, 6 weeks old, contained only a few viable cells, while 6-month cultures, maintained on the 4–m–l medium, usually gave luxuriant growth on fresh medium. Milk and litmus milk also were found to be suitable media for stock cultures.

Potato- or carrot-extract media gave various results for Spieckermann and Kotthoff (19), Stapp (20), and Savile and Racicot (16). We have found that potato and carrot extracts are quite satisfactory as media, even without additions, provided the pH is adjusted and maintained at about 7.0. Some extracts are too acid to obtain any growth without an adjustment of the pH (16).

The isolation of *Corynebacterium sepedonicum* from infected tubers or stems of potatoes is quite easy on any of the recommended media. On some media, however, like the 4–d or 4–m, the growth is so accelerated that the time necessary for colonies to develop large enough for satisfactory isolation is reduced from about 9 days to only 5. It has been reported that on the medium recommended by the laboratories of the U. S. Department of Agriculture (7) growth is also fast and abundant. The preparation of this medium seems, however, to be somewhat more complicated than that of the one here described. In plate I, figs. 1–5, are presented photographs of 10-day-old cultures on some of the tested media.

If the tubers are already invaded by soft-rot bacteria, isolation is often virtually impossible because *Corynebacterium sepedonicum* is overgrown by rapidly and abundantly growing colonies of *Erwinia carotovora* or similar soft-rot organisms. It has been found in preliminary experiments that the addition of separately steam-sterilized sodium dichromate to the sterile medium 4–m–l in concentration 1:20,000 completely inhibited the growth of *E. carotovora*, while *C. sepedonicum* grew only slightly less abundantly than on the medium without sodium dichromate. The addition of sodium dichromate made it less difficult to isolate *C. sepedonicum* from tubers in an advanced stage of decomposition due to secondary invasion by the soft-rot organisms. On agar plates with medium 4–m–l alone, colonies of the bacteria causing the secondary infection were found to be present in varying numbers, but no colonies of *C. sepedonicum* appeared (Pl. I, fig. 7). On the same medium with the addition of sodium dichromate 1:20,000 colonies of *C. sepedonicum* appeared in considerable numbers in most of the tested cases (Pl. I, fig. 6). The size of the colonies and the speed of their growth

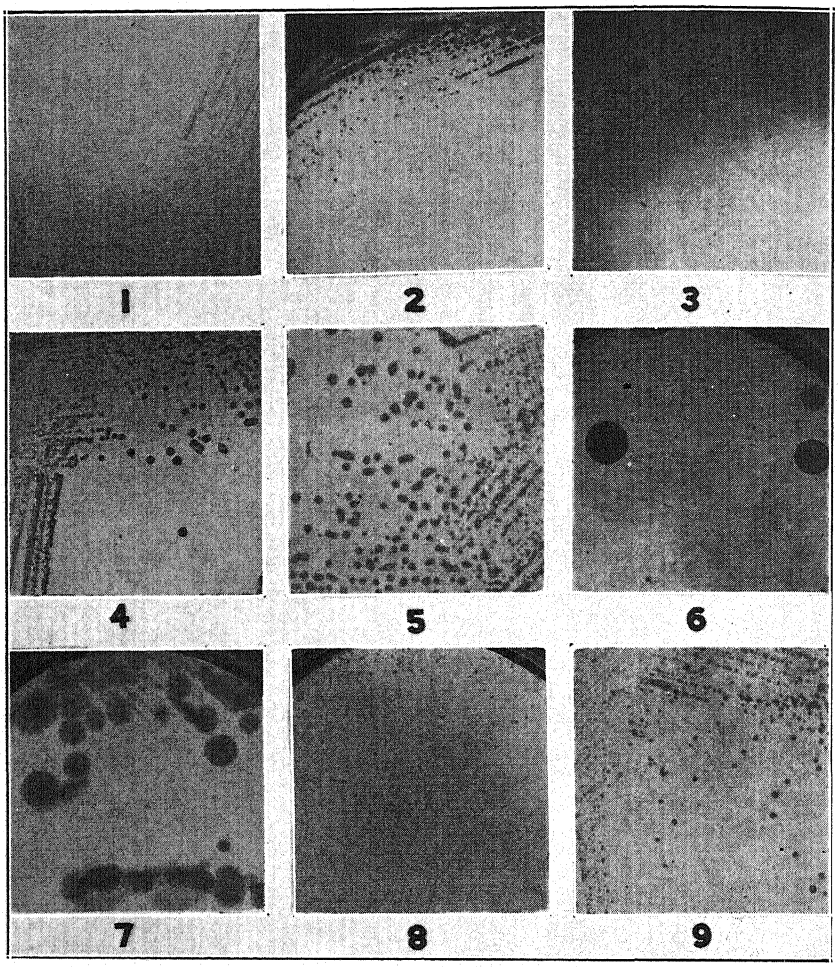



PLATE I

*Corynebacterium sepedonicum* cultures incubated 10 days at 22–23° C. FIG. 1. Nutrient agar plate. FIG. 2. Potato-extract agar plate with the addition of peptone, asparagine, and dextrose, pH 6.8–7.0. FIG. 3. Potato-extract agar plate, pH 6.8–7.0. FIG. 4. Medium No. 4-d agar plate, colonies of the "rough" type. FIG. 5. As figure 4, colonies of the "smooth" type. FIG. 6. Medium 4-m-1 agar plate inoculated with aseptically removed and macerated tissue of a potato tuber with the symptoms of late ring rot with the secondary symptoms of advanced soft rot. Medium contains sodium dichromate in concentration 1: 20,000. Colonies of *C. sepedonicum* fairly numerous with few large colonies of soft-rot organism. FIG. 7. The same as figure 6 but medium without sodium dichromate. Inoculated with the same material as in figure 6. Colonies of *C. sepedonicum* absent. Colonies of soft-rot organism numerous. FIG. 8. Medium 4-m-1 agar plate with the addition of sodium dichromate in concentration 1: 20,000. Inoculated with aseptically removed and macerated tissue of a potato tuber with early symptoms of ring rot without any secondary infection. Colonies of *C. sepedonicum* numerous but small. FIG. 9. The same as in figure 8 but medium without sodium dichromate. Colonies of *C. sepedonicum* well developed.

were less on the medium with dichromate than on the control medium without it (Pl. I, figs. 8, 9). This fact would indicate that at least a large proportion of the secondary invaders overcrowd not only because of greater numbers and faster growth but also because they exert a toxic effect on the *C. sepedonicum*. This toxic effect, however, apparently could be checked in the medium by the addition of small amounts of sodium dichromate.

Snyder and Lichstein (18) found that sodium azide is a bacteriostatic agent for Gram-negative bacteria, and Mallmann, Botwright and Churchill (14) demonstrated that both of the slowly oxidizing substances, namely, sodium azide and potassium dichromate, will inhibit the growth of Gram-negative bacteria and permit Gram-positive ones to grow. Wight (23) found that among various recommended slowly oxidizing agents, sodium dichromate had the widest range of growth inhibition for many Gram-negative bacteria.

### THE UTILIZATION AND FERMENTATION OF VARIOUS SOURCES OF CARBON

While cultivating various strains of *Corynebacterium sepedonicum* on media containing added sugars, it has been observed frequently that these media were distinctly acidified. Spieckermann and Kotthoff (19), who first studied the utilization of various sources of carbon by *C. sepedonicum*, found that very little, if any, acid was produced from the various sources of carbon, and that litmus added to the media remained unchanged in color. Stapp (20) made no mention of any acid production from various carbon sources by this organism. Because a difference was found between the reports of these authors and our own observations, a more detailed study was undertaken regarding the utilization and fermentation of various sources of carbon.

Medium 4 was used as a base, having no carbon sources added, as previously stated. A solid medium was prepared by the addition of 2 per cent of agar. Brom-thymol-blue, as the pH indicator, was added to the medium in the concentration of 0.002 per cent. The reaction of the medium was adjusted to approximately pH 7.0. Carbon sources were added in the concentration of 0.5 per cent. Those that would decompose in the autoclave were sterilized by filtration and added aseptically to the medium. Comparative observations were made on the cultures growing on solid media and in liquid media. Ten strains of *C. sepedonicum* were used, some several years old and the others isolated a few months or weeks before. Because the organism grew slowly, the fermentative changes in the medium frequently appeared very late.

Among all the tested sources of carbon (Table 1) it was mainly the monosaccharides that were fermented readily with distinct acidification of the medium. A medium containing dextrose was acidified faster when in the form of agar slants than as a liquid. The pH of the agar slants reverted within 20 days from 6.0 to 6.5 to 7.0, depending on strain. The pH of the liquid medium with dextrose remained near the 6.0 level until the close of

the experiment, or 50 days. A medium containing sucrose underwent nearly the same pH changes as the medium with dextrose. Maltose and lactose, also, were fermented, but more slowly; liquid media containing these sugars became acid within 50 to 80 days from inoculation, and in 100 to 130 days the acidity of the media gradually diminished. Of the alcohols tested, mannitol alone was fermented; the result was the production of acid in liquid medium within 40 to 50 days. Salicin was fermented only by the growth of the secondary colonies, which regularly appeared within 30 to 40 days of incubation. The resulting change of pH occurred suddenly due to the presence of an adaptive enzyme together with the sudden development of quite luxuriant growth of the secondary colonies on agar slants. In the liquid medium containing salicin the pH changes were less pronounced. It appeared that the secondary colonies were variants that were able to utilize salicin. The primary growth was scant, the same as on the check medium without any carbon source added. The secondary growth was abount as abundant as on the media containing any of the readily utilized sources of carbon. The two consecutive transfers from the secondary colonies to fresh medium with salicin failed to result in the accelerated fermentation of salicin pointing to the fact that the fermentative adaptation was only temporary.

### GROWTH AND VIABILITY IN LITMUS MILK

Particularly interesting was the type of growth in litmus milk. Fat-free milk with the addition of *lacmoid indica* was sterilized in live steam for 3 consecutive days, tested for sterility, inoculated with various strains of *Corynebacterium sepedonicum* and incubated at approximately 25° C. During the first 40 days of the incubation the only sign of growth was the reduction of the litmus for about 1 cm. from the bottom of the test tube. Between 40 and 60 days after inoculation, all strains, except one that was also serologically atypical, reduced litmus completely throughout the medium, and developed a transparent yellow serum zone 1 to 2 cm. deep on the top. Up to the end of 130 days of cultivation no other changes occurred, except for the slow widening of the serum zone. This zone was viscid and gelatinous, reminding one of the vaseline seal used on anaerobic media. It apparently reduces the evaporation of the milk, which evaporated less than half as fast as other liquid media. Jensen (13) found that such a zone is produced in milk by various saprophytic soil corynebacteria and mycobacteria, and is one of the very important differential characteristics of those microorganisms. The milk underneath at first appears coagulated; but, when the gelatinous serum zone is removed from the top, it is found to be normal and not coagulated. It has been found that litmus milk can be used successfully for the maintenance of stock cultures of *C. sepedonicum*. Litmus remained reduced for at least 8 months in the milk cultures of this organism left undisturbed at room temperature. In some of the cultures in which the gelatinous serum zone was broken up by shaking,

the litmus became oxidized. Transfers made sometime later from the milk cultures in which litmus was reduced gave very abundant growth, while transfers from cultures in which litmus became oxidized gave no growth, or reproduced only very few cells. It also has been found that when the number of viable cells of *C. sepedonicum* in litmus milk culture begins to decrease, the oxidation of the litmus in the top layer of the medium takes place. This is the best indication that the stock cultures should be transferred to a fresh medium. Stock cultures maintained in the liquid medium "4–l" (with lactose) survived nearly as well as those in litmus milk, while in liquid medium 4–m (with maltose) less than half of the strains survived during the same period of time and under the same conditions.

### SURVIVAL IN THE SOIL

There is a question whether or not *Corynebacterium sepedonicum* can survive the winter in the soil. Most American authors, Bonde (2, 3, 4), Eddins (9 and 10), Eide and Rose (11), Vaughn and Leach (22), Dykstra (7) and Dykstra *et al.* (8), claim that *C. sepedonicum* cannot overwinter in the soil. This conclusion has been based on the fact that potato seed pieces free from ring-rot infection, planted in soil in which infected plants formerly were grown, gave a healthy crop. Stapp (20) found that *C. sepedonicum* left buried in the field in soil, after inoculation on sterile media and introduction into sterile soil, survived from November to May. In our experiments pure cultures of *C. sepedonicum* were added to sterile samples of Caribou and Washburn loams. These samples were placed in 24 one-quart glass jars with glass tops and rubber gaskets; 400 g. of air-dry soil was put in each jar. To each alternate sample 10 g. of powdered calcium carbonate was added. The preparations were then sterilized for several hours at 20 pounds pressure and inoculated each with 25 ml. of a water suspension of *C. sepedonicum*. The amount of sterilized water then added to various jars varied from 25 to 100 ml. per jar. The jar clamps were closed and the jars placed in clay drainage tiles that were buried in the field at Aroostook Farm at a depth of eight inches beneath the surface of the soil on October 28, 1941. Upon removal in May, 1942, the soil in the jars was tested bacteriologically. Of the 24 jars buried in the soil, 15 were found to contain soil contaminated with various Gram-negative bacteria, which, being also non-spore-forming, obviously had penetrated the jars past the rubber gaskets (the jars not being vacuum sealed). The soil in 4 jars with Caribou loam was sterile, even *C. sepedonicum* was absent. The soil in 5 jars contained *C. sepedonicum* alone. Those 5 jars yielded cultures that were all identified as *C. sepedonicum* on the basis of their morphological, biological, serological, and pathogenic properties. The reisolated strains were tested in regard to their virulence and were found to be pathogenic causing typical ring-rot symptoms when used for inoculating potatoes. To 4 of these 5 soil samples calcium carbonate had been added. No definite influence or soil type or of water content of the soil was noticed.

TABLE 1.—*Utilization and fermentation of various sources of carbon*

| Carbon source | Utilization as estimated by the amount of growth[a] | | | Utilization as estimated by the change in pH on medium 4 (Snieszko and Bonde—1941–42) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | In liquid medium[b] | | | | | On agar slants |
| | Spiecker-mann and Kotthoff 1914 | Stapp 1930 | Snieszko, Bonde 1941–42 (within 10 days) | 10 days | 20 days | 30 days | 40 days | 50 days | (Medium 4 with sources of carbon) |
| l-Arabinose | ++++ | + | ++++ | +++ ++++ | ++++ | ++++ | ++++ | ++++ | pH 6.2–6.4 in ten days |
| Xylose | ++++ | + | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | pH below 6.0 in ten days |
| Rhamnose | ++++ | + | 0 | 0 | Slightly alkalized | | | | As check |
| Dextrose[c] | ++++ | ++++ | ++++ | ++ | +++ ++++ | ++++ | ++++ | +++ ++++ | pH 6.0 within ten days; within next ten days the pH of most strains slowly returned to 6.5–7.0 |
| Galactose | ++++ | ++++ | ++++ | ++ +++ | ++++ | ++++ | ++++ | ++++ | pH 6.4–6.6 in ten days; after 20 days returned to original pH |
| d-Levulose | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | pH 6.0 in ten days |
| Saccharose[c] | ..... | ++ | ++++ | 0 | +++ | +++ | ++++ | ++++ | pH 6.0 in ten days; in 40 days returned to 6.8 |
| Lactose[c] | ..... | ++ | + | 0 | + | + | ++ | ++[c] | As check |
| Cellobiose | ..... | ..... | +++ | + | ++ | +++ | ++++ | ++++ | pH 6.4–6.6 in 20 days |
| Maltose[c] | ..... | ++ | ++++ | + | ++ | ++ | ++ +++ | +++[c] ++++ | pH 6.5 in 20 to 30 days |
| Raffinose | ..... | + | 0 | 0 | 0 | 0 | Slightly alkalized | | As check |
| Inulin | ..... | + | 0 | 0 | 0 | 0 | Slightly alkalized | | As check |
| Starch, soluble | + | 0 | ± | 0 | 0 | 0 | Slightly alkalized | | As check |
| Dextrin | ..... | + | 0 | 0 | 0 | 0 | Slightly alkalized | | As check |
| Glycogen | ..... | 0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Ethyl alcohol | ..... | ..... | 0 | 0 | 0 | Slightly alkalized | | | As check |
| Glycerol | ++ | + | + | Some strains produced some acid; some strains were slightly alkalized | | | | | Some strains slightly alkalized, some slightly acidified |
| Adonitol | ..... | ..... | 0 | 0 | 0 | 0 | Slightly alkalized | | As check |
| d-Mannitol[c] | ..... | ++ | +++ | + | ++ | ++ | +++ | ++++ | Trace of acid in 10 to 20 days; later returned to original pH |
| Dulcitol | ..... | ++ | 0 | 0 | 0 | 0 | 0 | 0 | As check |
| Sorbitol | ..... | ..... | 0 | 0 | 0 | 0 | 0 | 0 | As check |
| Salicin[c] | ..... | ..... | + (+++[c]) | 0 | 0 | 0 | ++ | ++ | pH 6.0 or below in 30 to 40 days |
| Esculin[d] | ..... | ..... | + | ++ | ++ | ++ | ++ | ++[d] | Acidified in 10 days |
| Inosite | ..... | ..... | 0 | 0 | 0 | Slightly alkalized | | | As check |
| Check (medium 4 alone) | ..... | ..... | Growth scant | 0 | Slightly alkalized | | | | Slightly alkalized in 20 days |

[a] In these 3 columns, 0 = no growth, ++ = moderate growth, +++ = good growth, ++++ = abundant growth.

[b] In these 5 columns, 0 = no change in pH, or growth the same as in check, + = pH 6.8 to 7.0, ++ = pH 6.5 to 6.8, +++ = pH 6.2 to 6.5, ++++ = pH 6.0 or below.

[c] More details in text.

[d] pH not determined.

Data presented in table 2 show that those cultures of *Corynebacterium sepedonicum* reisolated from soil and agglutinated by the specific agglutinating serum gave high incidence of infection. Those that did not agglutinate were very slightly or not at all virulent.

This experiment is in agreement with the results obtained by Stapp (20); the fact, however, that *Corynebacterium sepedonicum* was isolated

TABLE 2.—*Pathogenicity of Corynebacterium sepedonicum reisolated from soil overwintered in the field*[a]

| Cultures reisolated from soil samples | | Infection occurring |
|---|---|---|
| *No.* | | *Per cent* |
| 10 | Morphological, biological and serological characteristics typical for *C. sepedonicum* | 90 |
| 11 | | 40 |
| 18 | | 100 |
| 20 | | 50 |
| 21 | | 100 |
| 6 | Morphological and biological characteristics typical, but serologically atypical | 0 |
| 14 | | 10 |
| 22 | | 10 |
| | Macerated tissue of the potato tuber with symptoms of ring rot | 100 |

[a] Pure cultures of *Corynebacterium sepedonicum* were added to sterilized soil in Mason jars. The jars containing the cultures were placed in clay tiles submerged in the field and then covered with eight inches of field soil. The cultures remained in the field from October 28 until May 25, and were then removed and taken to Orono where the organism was reisolated and grown on artificial media.

from none of the contaminated jars would suggest either that *C. sepedonicum* cannot survive long in the soil in the presence of other bacteria, or that the isolation of *C. sepedonicum* from a mixed bacterial flora was not possible due to the overcrowding of *C. sepedonicum* on the agar plates during the attempted isolation. This finding would be in agreement with the results obtained in Minnesota and presented by Dykstra (7), who reported that *C. sepedonicum* in nonsterile soil survived for a shorter time than in sterile soil. Data presented in table 3 also indicate that the ring-rot organism dies off faster in badly disintegrated tubers in which secondary invaders are present, than in tubers containing *C. sepedonicum* in a pure state.

Another indication that *Corynebacterium sepedonicum* cannot survive long in the presence of certain other microorganisms was obtained when a number of badly disintegrating potatoes were tested microscopically and culturally for the presence of the ring-rot organism. In all of the tubers tested *C. sepedonicum* was found to be present in preparations stained with the Gram method. Secondary invaders also were seen in the preparations in varying numbers, but the amount of *C. sepedonicum* was always vastly predominant. On the medium 4–m–l agar plates, with the addition of sodium dichromate 1 : 20,000 and without it, *C. sepedonicum* grew only in few cases, these being on the medium with the dichromate. The number of colonies of the contaminants on the medium with the dichromate was always moderate

and not sufficient to suppress entirely the growth of *C. sepedonicum*. This experiment, together with the infection experiments and the results reported by Dykstra (7), would indicate that *C. sepedonicum* dies quickly in the presence of other microorganisms.

TABLE 3.—*The effect of aging of ring-rot inoculum on its pathogenicity*[a]

| Inoculation number | Material used as inoculum | Seed pieces inoculated | Infected plants resulting from inoculation |
|---|---|---|---|
| | | *Number* | *Per cent* |
| 1 | Control. Macerated ring-rot tubers used immediately for inoculations | 10 | 100 |
| 2 | As No. 1 but stored 13 days | 10 | 0 |
| 3 | Badly disintegrated ring-rot tuber macerated in sterile water, used immediately for inoculation | 20 | 0 |
| 4 | Control. Macerated not disintegrated ring-rot tubers used immediately for inoculations | 20 | 70 |

[a] Studies conducted in the greenhouse.

## EXPERIMENTS ON VIRULENCE

On January 29, and July 1, 1942, cut potato seed pieces of the Green Mountain variety, which was found by Bonde *et al.* (5) to be very susceptible, were inoculated and planted. The inoculum was 10 strains of *Corynebacterium sepedonicum*, one half isolated in September of 1942 and the other half during 1940. Cultures grown 22 days on the solid medium 4–m were washed off with water to form suspensions in which the cut potato seed pieces were dipped shortly before planting. Potato plants were grown in a greenhouse at Presque Isle at the average temperature of 65° C. Observations were made on April 23, May 17, and September 11, 1942, and were based on the presence or absence of external symptoms of the disease. The results indicate (Table 4) that the cultures of *C. sepedonicum*, maintained for longer periods on artificial media, were losing their virulence markedly.

Between the results described in the two tests of table 4 the lapse of time was 6 months. It shows that all the cultures were losing their virulence markedly, even in such a short period. This fact may explain why certain workers have not secured successful results from inoculation with the cultures grown for a longer period on artificial media.

## SEROLOGICAL EXPERIMENTS

Two rabbits were immunized by repeated weekly intravenous injections of suspensions of *Corynebacterium sepedonicum*. No pathogenic effects were noticed in the rabbits, even after injecting into them large doses of the living bacteria. The agglutinating sera obtained, as in the case of nearly all Gram-positive bacteria, had low titre, namely:

Serum 1. 1:40 complete agglutination
1:80 partial agglutination

Serum 2. 1:5 complete agglutination
1:10 partial agglutination
1:20 partial agglutination

TABLE 4.—*Pathogenicity of strains of Corynebacterium sepedonicum*[a]

| Strain number[b] | Infection occurring[c] | |
|---|---|---|
| | First test[d] | Second test[e] |
| | *Per cent* | *Per cent* |
| 2 | 90 | 10 |
| 4 | 0 | 20 |
| 8 | 0 | 0 |
| 8a | 10 | 0 |
| 10 | 80 | ..... |
| 11 | 90 | 20 |
| 12 | 90 | 0 |
| 13 | 100 | 40 |
| 14 | 100 | 70 |
| 15 | 100 | ..... |
| Control; pulp from infected potatoes smeared on seed pieces | 70 | 100 |
| Control; water contaminated with bacteria from infected tubers | 87 | 100 |

[a] These strains had been studied also regarding their morphological and physiological properties.

[b] Strains 2, 4, 8, 8a, and 10 are older strains isolated by Bonde in 1940. Strains 11 to 15 were isolated by Snieszko in September, 1941.

[c] Ten freshly cut seed pieces of the Green Mountain variety were inoculated for each test by being dipped in water suspension of the bacteria and then planted immediately in the greenhouse. The bacteria were grown on agar in "French square" bottles, and the bacteria from each bottle were suspended in 200 cc. of tap water.

[d] The seed pieces were inoculated January 29, and the symptoms on the plants recorded May 17, 1942.

[e] The seed pieces were inoculated July 1, and the symptoms noted September 11, 1942.

All 19 strains of *Corynebacterium sepedonicum* isolated in the course of the last few years were tested. The test was made with the saline suspensions of the bacteria with an addition of 0.3 per cent of phenol. All tests were incubated for 48 hours at 25° C. The results were recorded after 12, 24, and 48 hours. The tests were actually completed within 12 to 24 hours, so that further incubation was proved to be superfluous. All suspensions were checked with the pure saline, normal rabbit and sheep sera, and the rabbit-agglutinating sera against entirely different bacteria belonging to the genus Salmonella.

None of the tested strains of *Corynebacterium sepedonicum* agglutinated with the physiological saline solution alone, two strains (7 and 8a) agglutinated with normal and heterologic sera, and one (No. 16) was not agglutinated by the specific sera. One strain (No. 4) was agglutinated by serum No. 1 in dilution 1:10 but not by serum No. 2. Strains 8a and 16 were somewhat atypical in their other properties also. The rest of the strains

were agglutinated approximately up to the titre of both sera. This would indicate that *C. sepedonicum* forms a quite uniform serological group, and that the agglutination test may be used for the identification of this bacterium.

Tipograf (21) described a serological method of diagnosis of the ring-rot organism. The description of the method found in an English abstract "does not seem reasonable," according to Burkholder (6). The vascular tissue of the potato tuber in the advanced stage of infection is filled with *Corynebacterium sepedonicum* and, therefore, it could be used as an antigen in the agglutination test. The tests made with the available sera did not, however, give any promising results, because in a macroscopic agglutination test floccules of tissue could not be distinguished from the agglutinated bacteria. In the microscopic agglutination test the differences between the tests and controls were so insignificant, if indeed there were any, that the test appeared to have no value.

Israilski and Artemieva (12) recommended as reliable the precipitation test for the diagnosis of tomato plants infected by *Corynebacterium michiganense*. However, the preliminary precipitation tests, which we made with the extracts of *C. sepedonicum*, gave negative results.

## TERMINOLOGY AND SYSTEMATIC POSITION OF CORYNEBACTERIUM SEPEDONICUM (SPIECKERMANN AND KOTTHOFF) JENSEN

Jensen (13) suggested that the bacterium causing the ring rot of potatoes should be clasified as a *Corynebacterium*. Skaptason and Burkholder (17) found that it "has all the characteristics of a *Corynebacterium.*" A study of the morphological and physiological characteristics of the bacteria, isolated from potatoes affected with ring rot, also has convinced the writers that the most proper systematic position of this organism is in the genus *Corynebacterium*. It has been found that the growth in milk is very typical with the production of the gelatinous serum zone that is considered by Jensen (13) to be an important characteristic. The only difference, a very insignificant one, between the properties of the strains of *C. sepedonicum* in our description and the description given by Stapp (20) is that none of our strains liquefied gelatine, while some of those freshly isolated by Stapp liquefied it slightly. Spieckermann and Kotthoff (19), on the other hand, did not find any liquefaction of gelatine.

The evidence at hand and the descriptions given by other authors support the view that the organism causing the ring rot of potatoes should be classified as *Corynebacterium sepedonicum* (Spieckermann and Kotthoff) Jensen.

## SUMMARY

A new medium is recommended for the isolation and cultivation of *Corynebacterium sepedonicum*. As nitrogen sources this medium contains Bacto peptone, Bacto tryptose and Bacto yeast extract, and as carbon sources, dextrose, maltose, and occasionally lactose.

For the maintenance of stock cultures, liquid media are more suitable than agar media. Litmus milk is a splendid medium for identification and for the cultivation of stock cultures.

The best sources of carbon are monosaccharides such as arabinose, xylose, dextrose, galactose and levulose; rhamnose is not utilized. Disaccharides are utilized more slowly. Among other sources of carbon, mannitol is the best. All sources of carbon which are utilized are decomposed with a slow and sometimes only temporary increase of the acidity of the medium.

The new medium with the addition of sodium dichromate in concentration 1:20,000 can be used for the successful isolation of *C. sepedonicum* from potato tubers having symptoms of soft rot due to secondary infection with *Erwinia carotovora.*

Pure cultures of *C. sepedonicum* when added to sterile soil and left buried in the ground during the winter survived and retained their virulence.

Cultures of *C. sepedonicum,* if maintained on laboratory media, gradually lose their virulence towards potatoes. Infection experiments in which macerated ring-rot tubers were stored for 13 days before inoculation gave negative results. Also, badly decayed tubers of ring-rot potatoes were not infectious.

*C. sepedonicum* is weakly antigenic for rabbits, producing agglutinating sera with low titre. All tested strains of *C. sepedonicum* were cross-agglutinated approximately up to the titre of all sera.

## LITERATURE CITED


1. BONDE, REINER. A bacterial wilt and soft rot of the potato in Maine. Phytopath. 27: 106–108. 1937.
2. ———. Bacterial wilt and soft rot of the potato. Amer. Potato Jour. 16: 109–114. 1939.
3. ———. Bacterial wilt and soft rot of the potato in Maine. Maine Agr. Exp. Stat. Bull. 396. 1939.
4. ———. Ring rot in volunteer plants. Amer. Potato Jour. 19: 131–133. 1942.
5. ———, F. J. STEVENSON, C. F. CLARK, and R. V. AKELEY. Resistance of certain potato varieties and seedling progenies to ring rot. Phytopath. 32: 813–819. 1942.
6. BURKHOLDER, W. H. Diagnosis of the bacterial ring rot of the potato. Amer. Potato Jour. 19: 208–212. 1942.
7. DYKSTRA, T. P. Compilation of results in control of potato ring rot in 1941. Amer. Potato Jour. 19: 175–196. 1942.
8. ———, R. W. GOSS, and J. G. LEACH. Report of the committee to coordinate research on new and unusual potato diseases. Amer. Potato Jour. 17: 81–88. 1940.
9. EDDINS, A. H. Some characteristics of bacterial ring rot of potatoes. Amer. Potato Jour. 16: 309–322. 1939.
10. ———. Bacterial ring rot of potatoes. Fla. Agr. Exp. Stat. Press Bull. 545. 1940.
11. EIDE, C. J., and R. C. ROSE. How to control bacterial ring rot. Minn. Agr. Ext. Serv. Folder 95. 1941.
12. ISRAILSKI, W. P., and S. S. ARTEMIEVA. Serologische Untersuchungen der durch die Bakteriose befallenen Pflanzen. III. Untersuchungen der Tomaten auf *Aplanobacter michiganense.* Microbiology 10: 74–80. 1941. Abst. in Rev. Appl. Mycol. 21: 172. 1942.
13. JENSEN, H. L. Studies on saprophytic Mycobacteria and Corynebacteria. Proc. Linn. Soc. New South Wales 59: 19–61. 1934.

14. MALLMANN, W. L., W. E. BOTWRIGHT, and E. S. CHURCHILL. The selective bacteriostatic effect of slow oxidizing agents. Jour. Inf. Dis. 69: 215–219. 1941.
15. RACICOT, H. N., D. B. O. SAVILE, and I. L. CONNERS. Bacterial wilt and rot of potatoes.—Some suggestions for its detection, verification, and control. Amer. Potato Jour. 15: 312–318. 1938.
16. SAVILE, D. B. O., and H. N. RACICOT. Bacterial wilt and rot of potatoes. Sci. Agr. 17: 518–522. 1937.
17. SKAPTASON, J. B., and W. H. BURKHOLDER. Classification and nomenclature of the pathogen causing bacterial ring rot of potatoes. Phytopath. 32: 439–441. 1942.
18. SNYDER, M. L., and H. C. LICHSTEIN. Sodium azide as an inhibiting substance for Gram-negative bacteria. Jour. Inf. Dis. 67: 113–115. 1940.
19. SPIECKERMANN, A., and P. KOTTHOFF. Untersuchungen über die Kartoffelpflanze und ihre Krankheiten. 1. Die Bakterienringfäule der Kartoffelpflanze. Landw. Jahrb. 46: 659–732. 1914.
20. STAPP, C. Beiträge zur Kenntnis des Bacterium sepedonicum Spieckerm. et Kotth., des Erregers der "Bakterienringfäule" der Kartoffel. Zeitschr. f. Parasitenk. 2: 756–823. 1930.
21. TIPOGRAF, D. Y. A rapid method of diagnosing ring rot of potato. C. R. Pan-Sov. V.I. Lenin Acad. Agr. Sci. 5: 35–38. 1941. Abst. in Rev. Appl. Mycol. 21: 40. 1942.
22. VAUGHN, J. R., and J. G. LEACH. Bacterial ring rot of potatoes. West Virginia Agr. Exp. Sta. Circ. 77. 1942.
23. WIGHT, K. M. The effect of slow oxidizing agents on bacteria. Bachelor of Arts Thesis, University of Maine. Unpublished. 1942.

# ACUTE AND CHRONIC SYMPTOMS IN TOBACCO MOSAICS

H. H. McKinney[1] and E. E. Clayton

(Accepted for publication April 30, 1943)

## INTRODUCTION

In the tobacco mosaics the disease reactions are not uniform in all leaves of a given plant. In 1929, McKinney (4) reported and illustrated the striking chlorotic reaction, referred to as the "oak-leaf" pattern by later workers. This appears in tobacco leaves advanced beyond a certain stage of development at the time of inoculation with a yellow-mosaic mutant from the virus of tobacco common mosaic. In 1932, Holmes (3) reported 5 symptom reactions in tobacco plants infected with the tobacco common-mosaic virus.

Studies on the ring-spot disease of tobacco led Valleau (13) and also McKinney (5) to take the view that the disease manifests an acute phase and a chronic phase, and that the so-called acquired immunity in this disease may be regarded as the chronic phase. McKinney (5) took the position that in the tobacco ring-spot disease, the low level of symptom expression in the chronic phase reflects natural resistance in the host. Pursuing this working hypothesis further, it became evident that the ring-spot virus is essentially a mosaic virus when it is studied in a more susceptible host (8). Accordingly, it was concluded that symptom reactions with tobacco mosaics should be reassessed, as many observations over a period of years indicated that acute and chronic disease reactions obtain in certain of these diseases also. Results of recent experiments on this point have been summarized by the writers (8).

This paper deals chiefly with the succession of symptoms occurring in the natural course of a yellow mosaic in tobacco; it gives also certain results of similar studies on common mosaic.

## MATERIALS AND METHODS

The host plants used were Samsun (Turkish) tobacco and one $F_1$ backcross generation (*Nicotiana tabacum* × *N. longiflora* × *N. tabacum*). The viruses used were wild-type common-mosaic virus (Nicotiana virus 1) (6), yellow-mosaic mutant virus *BSY* (6), and a yellow-mosaic virus (white-mosaic virus) (7) obtained from W. D. Valleau.

Except when otherwise stated, the inoculations were made with fresh native virus extract on a small amount of cotton inserted into the axil of a leaf near the base of the stem by means of a needle.

Tests have been carried out during all parts of the year, but the results summarized in figures 1, 2, and 3 are from tests conducted in a greenhouse during late summer and early autumn. Temperatures were held as near 22.5° C. as possible, but day temperatures usually were higher, as bright

[1] Acknowledgment is due Matthew Koerner for assistance in conducting the tests.

days prevailed during the test. Plants were grown in earthen pots of ample size. A composted soil was used throughout.

Throughout the paper, the term *acute* reaction denotes severe chlorosis and/or early death of the diseased tissue. The term *chronic* reaction denotes invaded tissue that may or may not express obvious symptoms, death

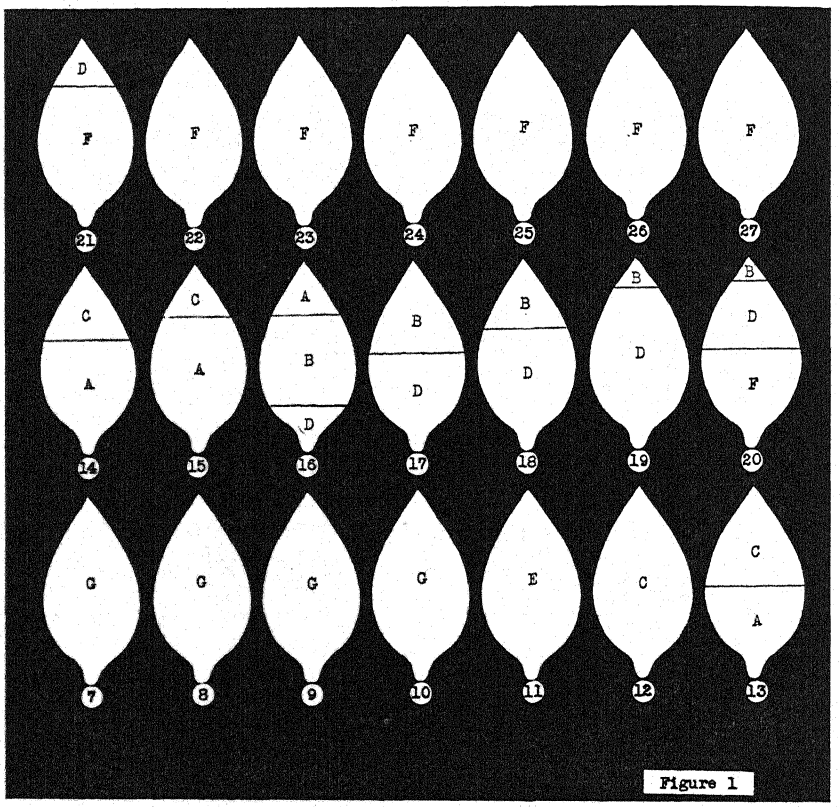



FIG. 1. Diagrams of mature leaves of virus-infected Samsun (Turkish) tobacco numbered from base of plant, to show the progressive appearance of the several symptom types in each leaf. Where two or more symptom types occur on the same leaf, their approximate boundaries are indicated by a line. The 7 symptom types are lettered A to G in the approximate order of their appearance. All leaves were made of uniform size in the diagram for convenience, but actually they were of different sizes as shown by the curve for mature leaves in figure 4. The symptom types are described on pages 1049 and 1050. Leaf numbers correspond with those in figures 2 and 3.

of tissues following chlorosis only after a long delay or under environmental conditions that are more extreme than those obtaining for the expression of acute reactions in the same plant.

## RESULTS

### Yellow Mosaic

In Samsun tobacco plants of moderate size the symptoms of yellow mosaic are summarized graphically in figure 1. Type symptoms are illustrated in

figures 2 and 3. In figure 4 the length of each leaf in mm. at the time of vein-clearing and at the time each leaf finished its growth, and the lengths of the mature leaves of a disease-free plant are graphed for comparison.

The several degrees of disease reaction or symptom types that appeared in the leaves assumed 2 gradient series. Proceeding from the most acute

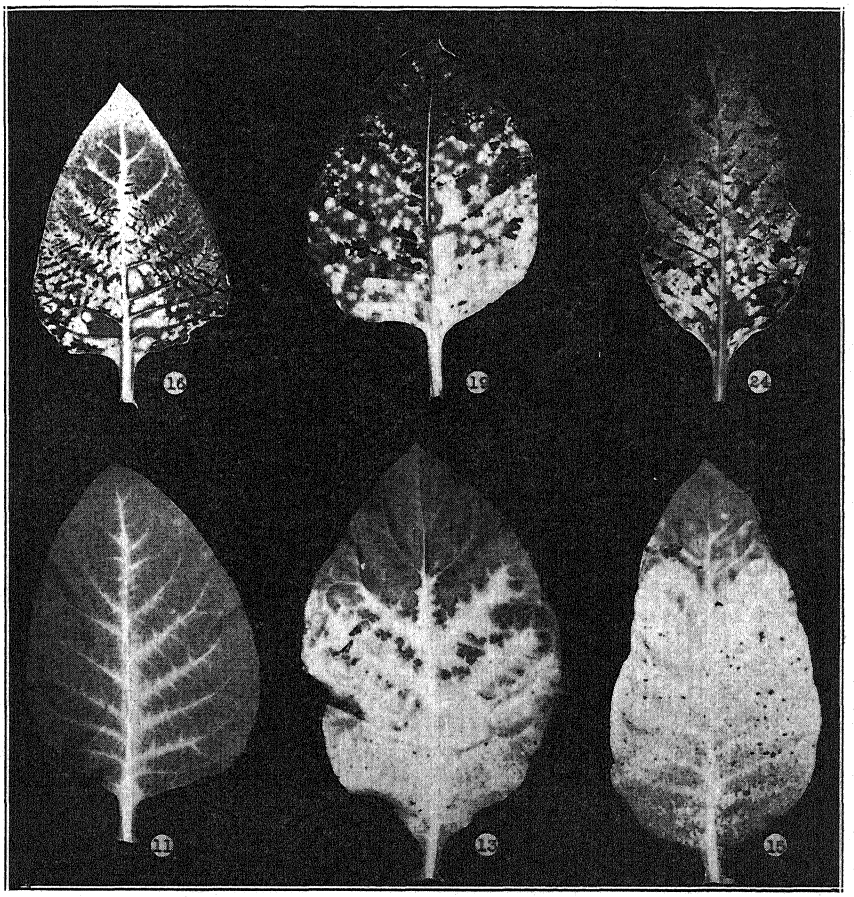


FIG. 2. Leaves of Samsun tobacco showing the types of symptoms referred to in the diagrams in figure 1. These leaves represent samples that correspond closely with the leaves diagrammed in figure 1, and the leaf numbers correspond with those in figure 1. Photographs were taken before necrosis appeared.

reaction (symptom type A), one gradient proceeded up, the other down the stem; progress being greater in the upward direction, especially during winter months. For sake of convenience, the entire series is divided into 7 rather characteristic zones of disease reactions or symptom types, and lettered A to G. Five types, designated A to E, inclusive, comprise the acute phase as follows:

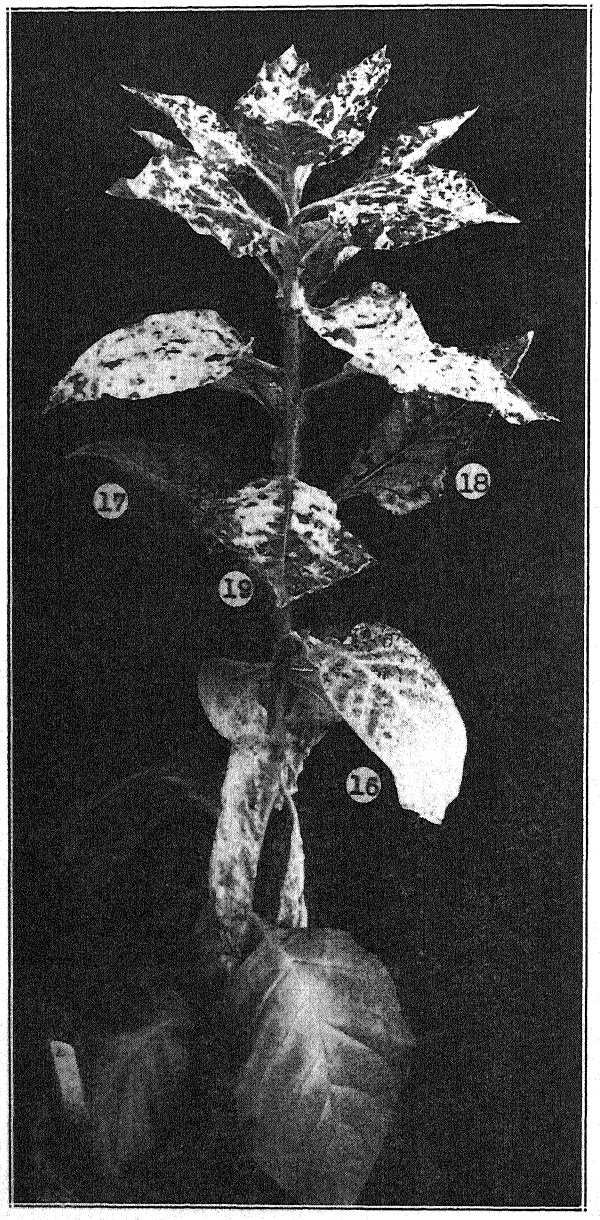



FIG. 3. Samsun tobacco plant with yellow mosaic. Leaf numbers correspond with those in figure 1. Necrosis and death occurred in leaves 12, 13, and 14, in tips of leaves 15 and 16, and in most of zone D in leaves 17, 18 and 19. Plant was inoculated in the stem near the base. $\times \frac{1}{4}$.

Symptom type A appeared first as vein clearing (faint chlorosis bordering the small vascular bundles). This was the first sign of disease in the plant, and it appeared approximately 8 days after inoculation, reaching its limits in about 8 hours, after which there was a rapid development of acute

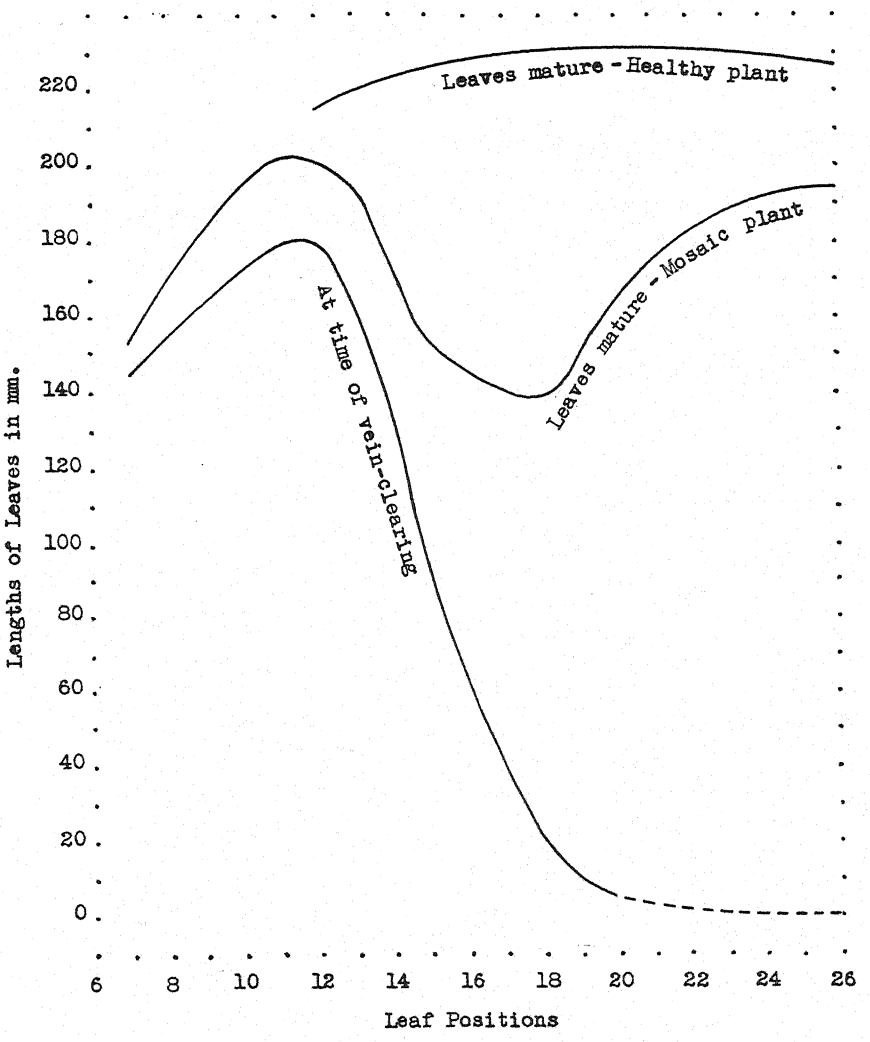



FIG. 4. Smoothed curves showing the length of leaves on a healthy Samsun tobacco plant and on a plant with yellow mosaic. The leaf position numbers correspond with those in figure 1.

chlorosis (severe yellowing) throughout most of the parenchyma within the vein-cleared zone. The original vein-cleared margins along the small veinlets tended to remain light-green, producing a netted effect illustrated in leaf 15 (Fig. 2). Acute necrosis was the final symptom. This represents the most severe reaction in the acute phase.

Symptom type B manifested no vein-clearing, but the development of severe chlorosis and acute necrosis resembled that in type A, except that wide light-green vein bands occurred along the large veinlets, as illustrated in leaf 16 (Fig. 2).

Symptom type C is characterized by chlorosis (severe yellowing), which progresses from the midrib, primary, secondary, and tertiary lateral veins, causing the "oak-leaf" pattern. Acute necrosis was characteristic (leaf 13, Fig. 2).

Symptom type D developed in very young tissue at the time chlorosis appeared in types A and B, and as type D developed, it tended to remain green for a considerable period, but later chlorotic and yellow patches appeared and caused the mottled effect illustrated in leaf 19 (Fig. 2). This reaction was followed by acute necrosis, especially in the yellow areas. This symptom type blends into the chronic-mosaic symptom type F.

Symptom type E is similar to type C, severe chlorosis occupied parenchyma tissue along the midrib, primary, secondary and tertiary lateral veins, acute necrosis was irregular (leaf 11, Fig. 2).

The chronic phase (typical mosaic mottling), represented by symptom type F (leaf 24, Fig. 2) continued in all subsequent leaves and there was no necrosis under minimal conditions that favored necrosis in the older leaves (types A to E). Typical mosaic commenced in leaf tissue that was differentiated near the time of vein-clearing. In growing leaves with the chronic yellow mosaic, the green mottled areas frequently developed "secondary" light-green and yellow areas as described in another paper (7), also the yellow areas usually took on a greenish color due to new cells that contained chlorophyll, or to the development of chlorophyll in cells that were devoid of it earlier. Leaves showing typical mosaic grew slowly and never reached the size of corresponding normal ones. During extremely hot, bright periods of mid-summer, necrosis sometimes was an attendant symptom in typical mosaic leaves that had attained about ½ or more of their growth. Necrosis in these leaves, however, is dependent on more extreme temperature and light conditions than it is in older leaves manifesting symptom types A to E.

After the onset of typical mosaic, the young leaves tend to exhibit the most intense mottling in the older tissues (distal region), and sometimes, especially during dark weather, more than one-half of the proximal portion of these leaves will be free of apparent symptoms. The progress of mottling in these areas resembles that occurring in tissue showing symptom type D, except that necrosis has not been identified as an attendant symptom.

Symptom type G manifests mild chlorosis, which spreads almost solidly from the primary lateral veins, involving all parenchyma as it progresses. Acute necrosis was not an attendant symptom; however, senility and final death of tissue was hastened. This may be regarded as a chronic manifestation in old leaves nearing their decline.

In young plants with the 8th leaf 42 mm. long at time of inoculation,

and also in younger plants, the acute chlorotic-necrotic stage was so severe that many plants were killed, and those that survived were very weak. Death seemed to result chiefly from the complete necrosis in leaves that exceed 10 mm. in length, as acute necrosis did not occur in the more resistant small leaves or in the tip of the stem.

Each disease reaction or symptom type tended to be associated with leaf tissue that had reached a particular stage of development at the time of infection. Chlorosis and necrosis occurred in acute form in leaves that ranged in length from near 179 mm. to 3 mm. (Nos. 12 to 21, inclusive, Figs. 1 and 3) at the time of vein-clearing. From the curves in figure 4, it will be observed that leaf 12 had attained about 83 per cent of its final length, as computed on a basis of the corresponding leaf on a healthy control plant. In the writers' abstract (8) this figure was given as 66.66 per cent. The former figure seems to be more nearly correct, but further study will doubtless result in a change in this value as the final accuracy of such comparisons depends on many observations. Some of the leaves manifested 2 and occasionally 3 types of disease reaction, and each type tended to be associated with tissues that had reached approximately the same age in the several leaves.

Individual plants show variations, and symptom types may occupy more or fewer leaves than are shown in figure 1, depending on the age of the plant at the time of inoculation, the amount of light, and the temperature after the onset of symptoms. Observations on several tests have indicated that the acute reactions are accentuated and hastened by bright sunlight and high summer temperatures and delayed and ameliorated by dark weather. No essential differences were noted between the two yellow-mosaic viruses used.

In certain interspecific crosses, the sequence and expression of symptoms induced by yellow-mosaic viruses of the *BSY* type differ from the situation in Samsun tobacco. One $F_1$ backcross generation (*Nicotiana tabacum* × *N. longiflora* × *N. tabacum*) obtained in 1941 is of special interest. At 21 to 24° C. the plants developed primary necrotic lesions abundantly on wiped leaves, a semi-mild form of secondary necrosis involved the midribs and/or the lateral veins of the wiped leaves and a few of the chronic-mosaic leaves. The portion of the stem cortex in the zone of the necrotic midribs of lower leaves also developed mild necrosis. Typical acute and chronic symptom types of yellow mosaic, similar to those in Turkish, occurred in all the plants. The progress of the necrosis was not increased when the plants were subjected to 31° C., and chronic yellow mosaic continued at 21 to 24° C. until the plants were matured.

When the chronic-mosaic leaves of these plants were wiped with the same virus employed in the initial inoculation (virus *BSY*), no signs of local necrosis or any other manifestation resulted. Thus it is apparent that the primary necrotic reaction that is characteristic of healthy leaves cannot

be induced in the chronic diseased tissue by reinoculation with the same virus.

### Common Mosaic

Distinction between acute and chronic symptoms is less striking in common mosaic than in yellow mosaic. The progress of the symptoms was slower than in the yellow mosaic, and this influenced the number of leaves in the acute phase, as well as the onset of the typical chronic symptoms.

Tobacco plants grown in the field frequently show necrosis in the older leaves when infection by common-mosaic virus occurs during midsummer. This acute reaction, referred to as blister by McMurtrey (10) and as burning by Valleau and Johnson (14), occurs rather commonly in infected plants, especially during hot weather.

### Virus Assays

The present study did not include extensive assays to determine the progress of virus in the tissues of the several symptom types. Preliminary assays, however, were made on certain tissues from yellow-mosaic plants, and the results are summarized.

When vein-clearing was just faintly evident in tobacco leaves 130 to 150 mm. long, small leaves 5 mm. long and on the same plants, were removed, weighed, pulped in a mortar and diluted 50 times in M/10 phosphate-buffered solution at pH 7. This inoculum was wiped on carborundum-dusted leaves on 5 Samsun (Turkish) tobacco plants. Since all of the test plants developed yellow mosaic it is evident that virus had reached the very small leaves by the time the first symptoms appeared in larger leaves. After the onset of the chronic yellow-mosaic symptoms, assays were made on (a) leaves ranging in length from 1 to 5 mm., (b) the proximal half of leaves 55 to 60 mm. long and (c) the distal half of leaves used in sample (b). No signs of mosaic mottling were present in sample (b), but there was much mottling in the tissue used in sample (c). All tissues were collected from the same plants. The samples were prepared as in the previous test and diluted to $10^{-4}$. Each sample was wiped on 20 primary leaves (10 plants) of *Phaseolus vulgaris* L., var. Scotia. Samples (a), (b), and (c) induced 2.7, 20.5, and 265.7 local lesions per plant, respectively. In a test reported previously (7) there was less virus activity in chronic-mosaic tobacco leaves 12 mm. long than in the larger leaves tested.

When vein-cleared leaf tissue had become almost completely chlorotic, but not necrotic, a comparison was made with leaf tissue at about the same stage of development, but with chronic-mosaic mottling. The leaves were from different plants. The tissues were processed as in the assays mentioned above, diluted to $2 \times 10^{-5}$, and the resulting inoculum was wiped on 20 primary leaves (10 plants) of Scotia beans. The vein-cleared chlorotic sample induced 21.6 lesions per bean plant, whereas the chronic-mosaic sample induced only 4.8 lesions per bean plant.

## DISCUSSION AND CONCLUSIONS

The results of these studies indicate that the natural course of tobacco mosaic in a given plant of Samsun (Turkish) involves two major phases, an acute phase and a chronic phase. The acute phase comprises several intergrading types of expression, and all expressions tend to form an unbroken and natural sequence that seems to parallel more closely the natural growth phases occurring in the developing leaves, than it does virus movement with respect to vascular channels in relation to leaf phylotaxy.

On the basis of the several degrees of severity of the disease reactions in relation to the age of the leaf tissues, it seems apparent that natural resistance changes with the growth and development of the leaf tissues, and that the level of this resistance, at the time the tissues become infected, tends to determine the type of disease reaction.

Leaf tissues in the early stages of development and those that have reached maturity are regarded as more resistant than tissues in the intervening stages. The chronic-mosaic phase appears in leaves that are differentiated at about the time vein-clearing becomes evident and thereafter. These leaves become infected when they are very small. It is possible that the initial cells are infected when leaf differentiation takes place, as Sheffield (12) found virus in the primordial meristem dissected from the stem apices of tomato plants infected with tobacco common-mosaic virus and with Aucuba-mosaic virus, respectively. Resistance in the young tissues may be due to inability to maintain a high level of virus synthesis and/or to some condition that tends to retard the movement of virus.

It is well known that leaf tissues differing in age differ also in their physiology; it, therefore, is not surprising that they react differently to a virus. With the onset of disease, new physiological balances are set up (1, 2, 9, 11, 15, 16, 17) that, in some cases, may influence the symptom reactions of subsequent tissues. Furthermore, it seems highly improbable that a normal physiologic balance ever obtains in infected tissues, even though gross symptoms may be absent (2, 11). It is to be expected, therefore, that the several disease reactions occurring throughout the course of a disease will maintain a natural sequence that is essentially fixed for a given host, virus, and environment; and that necrosis will not occur in chronic diseased leaves when an additional dose of the virus already present is added.

Divisions of Cereal Crops and Diseases and Tobacco Investigations,
Bureau of Plant Industry, Soils, and Agricultural Engineering,
Agricultural Research Administration,
U. S. Department of Agriculture

## LITERATURE CITED

1. Dunlap, A. A. The total nitrogen and carbohydrates, and the relative rates of respiration in virus-infected plants. Amer. Jour. Bot. 17: 348–357. 1930.
2. Hills, Claude H., and H. H. McKinney. The effect of mosaic virus infection on the protein content of susceptible and resistant strains of tobacco. Phytopath. 32: 857–866. 1942.

3. HOLMES, F. O. Symptoms of tobacco mosaic disease. Contrib. Boyce Thompson Inst. 4: 323–357. 1932.
4. MCKINNEY, H. H. Mosaic diseases in the Canary Islands, West Africa and Gibraltar. Jour. Agr. Res. [U.S.] 39: 557–578. 1929.
5. ————. Virus antagonism, natural host resistance, and the acquired-immunity concept with reference to plants. Phytopath. 31: 1059–1061. 1941.
6. ————. Virus-antagonism tests and their limitations for establishing relationship between mutants, and nonrelationship between distinct viruses. Amer. Jour. Bot. 28: 770–778. 1941.
7. ————. Reaction of resistant tobaccos to certain strains of Nicotiana virus 1 and other viruses. Phytopath. 33: [In press]. 1943.
8. ———— and E. E. CLAYTON. Acute and chronic symptoms in virus diseases. (Abst.) Phytopath. 33: 8. 1943.
9. ———— and CLAUDE H. HILLS. Mosaic, chlorosis and necrosis in virus-infected perennial pepper caused by products of a deranged metabolism. Science 94: 372–373. 1941.
10. MCMURTREY, J. E. Effect of mosaic disease on yield and quality of tobacco. Jour. Agr. Res. [U.S.] 38: 257–267. 1929.
11. MARTIN, L. F., A. K. BALLS, and H. H. MCKINNEY. Protein changes in mosaic-diseased tobacco. Jour. Biol. Chem. 130: 687–701. 1939.
12. SHEFFIELD, F. M. L. Presence of virus in the primordial meristem. Ann. Appl. Biol. 29: 16–17. 1942.
13. VALLEAU, W. D. Experimental production of symptoms in so-called recovered ring-spot tobacco plants and its bearing on acquired immunity. Phytopath. 31: 522–533. 1941.
14. ————, and E. M. JOHNSON. Burning and non-burning strains of tobacco mosaic. Kentucky Agr. Exp. Stat. Bull. 361, 233–238. 1935.
15. WOODS, ALBERT F. The destruction of chlorophyll by oxidizing enzymes. Centralbl. f. Bakt. u. Par., II Abt. 5: 745–754. 1899.
16. WYND, F. LYLE. Certain enzymatic activities of normal and mosaic-infected tobacco plants. Journ. Gen. Physiol. 25: 649–661. 1942.
17. ————. Respiration of mosaic-infected tobacco plants. Plant Physiol. 18: 90–98. 1943.

# USTILAGO STRIAEFORMIS. II. TEMPERATURE AS A FACTOR INFLUENCING DEVELOPMENT OF SMUTTED PLANTS OF POA PRATENSIS L. AND GERMINATION OF FRESH CHLAMYDOSPORES[1]

K. W. KREITLOW[2]

(Accepted for publication April 1, 1943)

## INTRODUCTION

Observations made in 1941 and 1942 showed that many plants of *Poa pratensis* L. in Pennsylvania pastures were infected with stripe smut *Ustilago striaeformis* (Westd.) Niessl. The extent of damage caused by this fungus is unknown; the disease, however, apparently is widespread, since practically every one of scores of pastures examined contained smutted plants.

The smutted plants, compared with healthy ones, were much reduced in size and seemed more adversely affected by the dry, hot mid-summer weather, when many of the diseased plants apparently die. Hence, it is to be expected that the heavy infestations of smut found in some pastures would result in considerable reduction in forage yields, particularly during mid-summer.

Since stripe smut is systemic and the causative organism may possibly be soil-borne, one of the most promising methods of control would seem to be in the selection or breeding of resistant varieties. The success of a breeding program is partly conditioned by the certainty with which infection can be produced in susceptible plants. A serious limitation to the approach of this problem is the frequent failure of fresh chlamydospores to germinate. The investigation here reported was undertaken to find, if possible, a treatment to hasten spore germination and determine the influence of temperature on development of smutted plants.

Examination of the literature revealed conflicting reports regarding germinability of the smut chlamydospores. Liro (5), inoculated different grasses with the organism but made no mention of encountering difficulties in securing germinable spores. Davis (1), stated that collections of *Ustilago striaeformis* obtained in May from *Poa pratensis* and *P. debilis* Torr. germinated without an after-ripening period; and Fischer (3) obtained 85 per cent germination of the chlamydospores from a collection of *U. striaeformis* on *P. pratensis* from Arlington, Virginia. On the other hand, collections of smut on *P. pratensis* tested by W. H. Davis (2), failed to germinate unless they were first subjected to an after-ripening period of 110–250 days. Germination of chlamydospores so treated, however, averaged only 35 to 60 per cent.

[1] Contribution No. 50, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the Northeastern States.

[2] Associate Pathologist.

Pammel (6), reported that chlamydospores obtained from *Phleum pratense* L. germinated readily. Fischer (3), encountered no difficulty in germinating spores of a race of *Ustilago striaeformis* that occurs on *Agropyron trachycaulum* (Link) Malte, and *Elymus glaucus* Buckl. From chlamydospores of this race, he obtained cultures of *U. striaeformis* on artificial media. Kreitlow (4), recently reported a collection of smutted *Agrostis alba* L. that yielded fresh, germinable chlamydospores. Cultures secured from single chlamydospores growing on artificial media differed from those obtained by Fischer.

## MATERIALS AND METHODS

Plants of *Poa pratensis*, infected with stripe smut, were collected from more than 75 pastures in Pennsylvania in 1941 and 1942. All diseased plants were separated into single tillers and transplanted to 4-inch pots of soil in a greenhouse. Chlamydospores from the smutted plants collected in each pasture were tested for germinability as soon as possible following collection.

Microscope slides that contained 4 paraffin wells were used in all germination tests (4). A drop of distilled water was placed within each of the 4 wells on a slide and chlamydospores of the organism were dispersed in the drops of water. Each slide was then placed in a Petri dish moist chamber and the chlamydospores were incubated 16 to 24 hours at room temperature.

## FIELD OBSERVATIONS

Plants of *Poa pratensis* infected with *Ustilago striaeformis* were secured from pastures in Pennsylvania as early as May 5. Diseased plants were easily located from early in May until the middle of June. Later a combination of factors that included high temperature, reduced growth, and close grazing made collecting of smutted material more difficult. After the advent of hot weather, leaves infected with smut curled and withered prematurely. Premature drying of smutted plants may be severe enough to produce conspicuous patches of dead leaves. This is illustrated in figure 1, showing a clonal plot of Kentucky bluegrass with an area heavily infested with smut. Smutted plants maintained in pots both in a greenhouse and outside followed a similar course of decline. This condition continued until fall, when cooler weather once more provided an environment more favorable for growth of bluegrass.

Smutted plants of *Poa pratensis* were collected from pastures until November 15, when low temperatures prevented new growth of diseased leaves and made the plants extremely difficult to locate. Nevertheless, presence of smutted plants was demonstrated in the sods of the pastures examined. Plants in sod plugs removed from parts of pastures judged free of the disease often developed smutted shoots when the plugs were maintained at favorable temperatures in a greenhouse. When suspected diseased

plants were transplanted to a greenhouse for observation, many later developed typical smutted shoots. This suggested the possibility that smut infection among plants of *P. pratensis* may be far more extensive than is generally realized.

Many smutted plants succumb during periods of hot, dry weather. That death of smutted plants is not due to drought alone during such periods was illustrated by an observation made in the summer of 1942. More than 200 pots of smutted Kentucky bluegrass were moved from inside a greenhouse to a bed of sand outdoors. Each pot containing a diseased plant growing in soil was buried to the rim in sand. The plants were watered

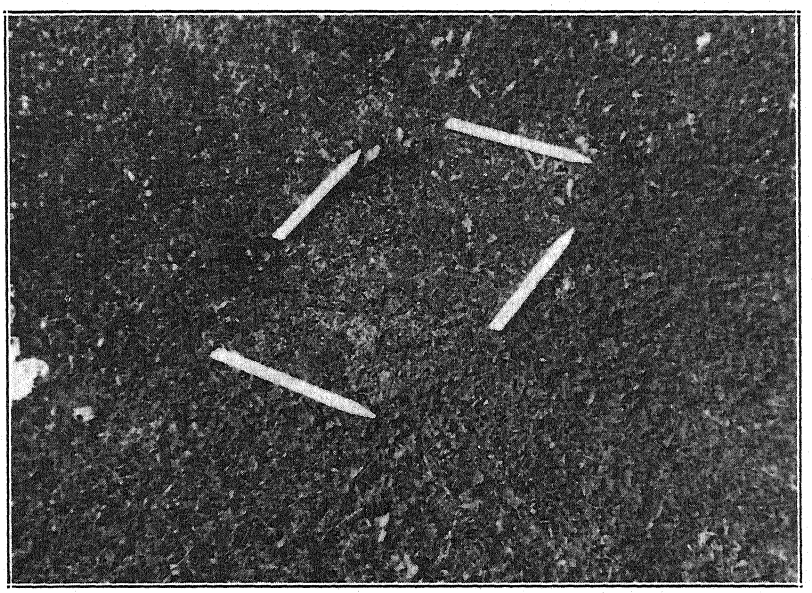


FIG. 1. Smutted plants of Kentucky bluegrass in a clonal plot in the nursery.

several times daily and shaded by a greenhouse from direct sunlight a part of each afternoon. Nearly one-half the plants died despite adequate moisture and protection from long exposure to sunlight. There also was a differential response of the plants from different collections; some continued to grow slowly throughout the summer, while others succumbed after a short time. Although the dead plants were not examined further, it seemed possible that they were so weakened by stripe smut as to be unduly predisposed to attacks by root-rotting organisms.

## INFLUENCE OF DIFFERENT TEMPERATURES ON DEVELOPMENT OF DISEASED PLANTS

Smutted plants were grown at different temperatures under controlled conditions to test the relationship of this factor to the observed fluctuations in development of diseased plants. For this purpose, a refrigerated dis-

play case in a greenhouse provided the necessary temperature chambers. The display case was divided into 3 compartments each of which was designed to maintain independent, thermostatically controlled temperatures. Heating elements in each of the chambers provided the higher temperatures when desired.

Single tillers from each of 23 different smutted clones were transplanted to 4-inch pots of soil. After the young diseased plants were well established, a representative plant from each of the 23 clones was placed in the chambers at temperatures of 1.5, 10, and 32° C., and a control series of plants was kept at 20 to 25° C. in the same greenhouse that contained the temperature-control chambers. The plants were watered twice each day and were under observation during 5 months.

The smutted plants maintained at 1.5° C. for 5 months grew slowly with no visible diminution in amount of smut. Plants growing at 10° C. were vigorous and developed numerous long leaves all of which were diseased but remained green. The plants that were growing at 20 to 25° C. also were vigorous but many of the severely infected leaves turned yellow and died. Of the 23 smutted clones tested at 32° C., only 14 were alive after 4 months. Most of the leaves, present when the plants were placed at this temperature, died shortly thereafter. New leaves that emerged were long and very narrow, so that the smut spores developed within small, separated sori scattered the length of a leaf. New shoots that developed from diseased plants often produced leaves that showed no visible symptoms of smut infection.

Plants that had survived exposure to 32° C. for 4 months were removed from the temperature chamber and placed on a greenhouse bench at 20 to 25° C. At that time 9 of the 14 plants displayed no visible evidence of smut infection. After the plants had remained at the lower temperature for 1 month they were again examined for smut. Only 10 of the 14 still survived, 4 of the smutted ones having succumbed. Of the 10 remaining plants, 4 showed no evidence of smut; the other 6 bore 1 to several diseased shoots that had emerged among the smut-free tillers. When the plants were examined again after 3 months' growth at 20 to 25° C., 3 plants remained smut-free.

The results of this experiment indicate that the temperature extremes endured by the disease organism are similar to those endured by the host plant. Optimum development of the host occurred at temperatures that were favorable for growth of the smut organism. The critical maximum temperature for survival of diseased plants was approximately 32° C. This temperature was slightly less favorable for development of *Ustilago striaeformis* than for the diseased plants, since part (9 out of 14) of the treated plants recovered temporarily from all visible symptoms of smut infection. The gradual reappearance of smut in the plants that seemed to have recovered indicated probably that the fungus had remained alive in the plants but was unable to cause disease symptoms at the high temperature.

### GERMINATION OF SMUT CHLAMYDOSPORES FROM PLANTS GROWING AT DIFFERENT TEMPERATURES

Davis (1) and Fischer (3), both secured germinable chlamydospores from diseased plants of *Poa pratensis* without first subjecting the spores to an after-ripening period. Since both authors worked with material collected in May, there are 2 possible explanations for their results; both authors chanced upon races of the organism that produced germinable chlamydospores or the environmental conditions that prevailed during May influenced, in some manner, the germinability of fresh chlamydospores.

In order to investigate the first possibility, germination tests were conducted on chlamydospores from diseased plants collected at different times during the growing season in widely separated pastures. Chlamydospores from more than 300 different smutted plants were examined, but none of the tests yielded more than an occasional germinated spore. The absence of germinable spores within sori on the diseased plants examined leads one to conclude that races of *Ustilago striaeformis* from *Poa pratensis* that possess germinable, fresh chlamydospores are rarely encountered, at least in central Pennsylvania.

Chlamydospores from diseased plants collected at frequent intervals from May to November showed no seasonal relationship to germinability. Additional germination tests were conducted, however, on chlamydospores from diseased plants growing at different controlled temperatures in the greenhouse. Fresh chlamydospores from plants grown at 32° C. for 3 months germinated as much as 90 per cent. Each of the surviving plants held at 32° C. yielded highly germinable chlamydospores even when the spores were removed directly from fresh green leaves. Spores from dead leaves on the same plants gave substantially lower germination percentages. Subsequent tests demonstrated that smutted plants, exposed to a temperature of 32° C. for as short a period as 2 weeks, yielded highly germinable chlamydospores.

In comparison with the results at 32° C., no germination was secured from fresh chlamydospores removed from the plants growing at 1.5, 10, and 20 to 25° C. These results indicated that the development of smut spores at relatively high temperatures, in some manner, influenced their germinability. Thus, there is the possibility that high temperatures also may influence germinability of fresh chlamydospores in the field.

### EFFECT OF TEMPERATURE ON LENGTH OF AFTER-RIPENING PERIOD OF SMUT CHLAMYDOSPORES

Davis (2), earlier reported that smut chlamydospores from leaves of *Poa pratensis* required an after-ripening period of 197 days in the laboratory. Since highly germinable fresh chlamydospores were obtained from smutted plants maintained at 32° C., the possibility was considered that a similar temperature treatment might stimulate germination of chlamydospores in detached smutted leaves. Diseased leaves were, therefore, removed from

plants growing in a greenhouse at 20 to 25° C. and placed in Petri dish moist chambers in an incubator at 35° C.

In preliminary tests, excellent germination occurred 30 days after the chlamydospores were placed at 35° C. Fresh smutted leaves from a number of different clones of *Poa pratensis* were then placed in moist chambers and incubated at the same temperature. At periodic intervals, germination tests were conducted on spores from the treated leaves.

As shown in table 1, very few germinable spores were found before the incubation period was started. After five days at 35° C., most of the treated

TABLE 1.—*Per cent germination of chlamydospores of Ustilago striaeformis from Poa pratensis incubated at 35° C.*

| Collection | Number of days incubated | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 25 | 30 |
| KB14E(2) | 0 | 0–few | few–10 | 25–75 | 30–50 | 0–few | ..... |
| KB36G(1) | 0 | 50–75 | 10 | 10–50 | 0–few | few–10 | ..... |
| KB34J(1) | 0–few | 0 | 25–35 | 0 | 0 | 10–25 | ..... |
| KB32A(1) | 0 | 0–few | 25–30 | 50 | 25–30 | 0–10 | ..... |
| 37KB120(26) | 0 | 0–few | 25–50 | 75–80 | 30–75 | few–25 | ..... |
| KB39K–2–7 | 0 | few | 50 | 30 | 10 | 10 | ..... |
| KB39G–2–2 | 0 | 0–few | 10 | 75 | 25 | 75 | ..... |
| KB21F–3–3 | 0 | 0 | ..... | ..... | 65–80 | ..... | ..... |
| KB3C–1–3 | 0 | few–10 | ..... | ..... | 80 | ..... | 30 |
| KB39G–2–3 | 0 | 0 | ..... | ..... | 10–90 | ..... | ..... |
| KB31E–1–3 | 0 | 0–10 | ..... | ..... | 50–90 | ..... | ..... |
| KB39G–1–4 | 0 | 0 | ..... | ..... | 50 | ..... | 50–75 |
| KB39L–1–3 | 0 | 0 | ..... | ..... | 75 | ..... | 80–90 |
| KB3B–1–5 | few | few–25 | ..... | ..... | 75–90 | ..... | ..... |
| KB21F–2–3 | 0–10 | 0–few | ..... | ..... | 10–25 | ..... | ..... |

samples yielded some germinable spores. Nearly all of the samples responded to the temperature treatment after 10 days incubation and most of the samples provided maximum numbers of germinable spores between the 10th and 20th days after treatment was started. After 20 days at 35° C., the percentage of germinating spores in each sample decreased progressively although there were appreciable numbers in some samples even after 30 days of treatment. Prolonging the incubation period beyond 30 days usually caused a pronounced decrease in percentage of germination. In tests of several collections maintained at 35° C. for 60 days the spores lost their ability to germinate despite the fact that 80 per cent of them were still germinable after 30 days.

Chlamydospores from collection KB34J(1) varied in germinability from one test to another. This is interpreted on the basis that considerable variation may exist in the state of development of chlamydospores from sori on the same or different leaves.

In a subsequent test of the effect of different temperatures, smutted leaves from each of 7 different plants of *Poa pratensis* were placed in Petri-dish moist chambers and incubated at 25, 30, 35, 40, and 45° C. At periodic intervals, germination tests were conducted on spores removed from the diseased material stored at each temperature.

Temperatures of 30 and 35° C. were found the most favorable for after-ripening the smut chlamydospores. Best germination of spores occurred when the smutted leaves were incubated at these temperatures for 10 to 30 days. There was very little germination of spores incubated at 40 and 45° C., while those incubated at 25° C. either germinated poorly or required a prolonged treatment. From the results obtained, a temperature treatment of 30 to 35° C. for 10 to 30 days seemed most favorable for after-ripening the smut chlamydospores.

### GERMINATION OF SMUT CHLAMYDOSPORES IN WATER AT DIFFERENT TEMPERATURES

Davis (2) reported that chlamydospores from diseased plants of *Poa pratensis* germinated in water at a minimum temperature of 7° C. and a maximum temperature of 35° C. A temperature of 22° C. was reported as optimum. In the present investigation, the germination tests were conducted as previously described and the slides with test drops of distilled water and spores were placed at temperatures ranging from 5° to 45° C. at 5° intervals. Spores that had been treated previously at 35° C. were used. Results of the tests on spores from 3 clones are shown in table 2. The temperatures at which the spores germinated agree substantially with those reported by Davis. The variation among cultures with respect to the temperature at which germination occurred may possibly be attributed to differences between strains of the organism or of the host plant.

TABLE 2.—*Per cent germination of smut chlamydospores of Ustilago striaeformis from Poa pratensis in water at different temperatures*

| Collection | Germination temperature | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5° C. | 10° C. | 15° C. | 20° C. | 25° C. | 30° C. | 35° C. | 40° C. | 45° C. |
| KB31F–1–3 | 0–few | few | few–10 | 15–25 | 50–80 | 80 | few | 0 | few |
| KB31E–1–3 | 0 | 0–few | few–10 | 50–70 | 80 | 80 | 75–80 | 0–few | 0 |
| 37KB120(26) | 0 | 0–few | 5–10 | 5–10 | 5–35 | 35–85 | 0 | 0 | 0 |

### DISCUSSION

The influence of temperature on development of smutted plants was demonstrated by field observations and greenhouse experiments. The ease with which smutted plants are found at certain seasons is correlated with the temperatures that prevail at that time. Cool periods in spring and fall are most favorable for development of *Poa pratensis*. From greenhouse tests, a continuous controlled temperature of 32° C. proved maximum for survival of smutted plants. Temperatures of 1.5, 10 and 20 to 25° C. were more favorable for their growth.

When smutted plants were grown for long periods at 32° C., new emergent leaves frequently failed to display symptoms of smut infection. If

these plants were removed to a lower temperature and permitted to grow for a time, smut symptoms, with a few exceptions, again appeared. These results indicated that prolonged exposure to high temperatures was more unfavorable to the disease organism than to the host. The smut organism, however, was not completely purged from the host by this treatment; so that, for the most part, restoration of favorable environmental conditions once more permitted the smut hyphae to become established throughout the plant.

The discovery that smutted plants maintained at 32° C. yield highly germinable chlamydospores offers a possible solution to the discrepant reports in the literature regarding germinability of fresh spores of *Ustilago striaeformis*. Two authors who reported germinable chlamydospores of this organism mentioned that the collections examined were secured from the field in May. It is not unusual for temperatures to approximate 32° C. for several days at a time in the latitudes represented by these reports. Similarly, chlamydospores from smutted plants collected in other areas might escape the length of exposure to high temperatures necessary to make the spores germinable.

The fact that smutted plants of *Poa pratensis* growing at a temperature of 32° C. yield highly germinable chlamydospores does not exclude the possibility that races of the organism with germinable chlamydospores may exist. To date, however, germination tests on fresh chlamydospores from smutted plants collected in more than 75 different pastures have failed to reveal races of the organism with germinable fresh spores.

A temperature of 30 to 35° C. was found most desirable for shortening the after-ripening period of smut chlamydospores in detached smutted leaves. Usually, an after-ripening period of 10 to 30 days proved sufficient for stimulating maximum germination of the spores. Prolonged high temperature, however, apparently reduced the viability of treated spores, since a progressive decrease in numbers of germinable spores resulted when the samples were incubated more than 30 days.

Chlamydospores, incubated at temperatures below 30° C., required a considerably longer period to after-ripen, while spores incubated at temperatures above 35° C. failed to germinate, even after prolonged treatment. The results of the experiments reported herein demonstrate that temperature is a prime factor in determining the length of time necessary to after-ripen chlamydospores of *Ustilago striaeformis* from *Poa pratensis*.

## SUMMARY

High temperatures in the field and in greenhouse experiments were unfavorable for development of *Poa pratensis* infected with *Ustilago striaeformis*.

Part of the smutted plants exposed to a continuous temperature of 32° C. lost their smut symptoms after 4 months, but, with a few exceptions, regained them when the plants were grown at a lower temperature.

Chlamydospores removed from leaves of smutted plants growing at 32° C. proved highly germinable, without any after-ripening period.

The after-ripening period of chlamydospores of *Ustilago striaeformis* was reduced from 200 days to less than 30 days by incubating detached smutted leaves in a moist chamber at 35° C.

Examination of chlamydospores from more than 300 smutted plants of *Poa pratensis* collected in 75 different pastures failed to reveal the presence of races of the organism with germinable fresh spores.

## LITERATURE CITED

1. DAVIS, J. J. Third supplementary list of parasitic fungi of Wisconsin. Trans. Wis. Acad. of Sci., Arts, Letters **14**: 83–106. 1902.
2. DAVIS, W. H. Summary of investigations with *Ustilago striaeformis* parasitizing some common grasses. Phytopath. **25**: 810–817. 1935.
3. FISCHER, GEORGE W. Fundamental studies of the stripe smut of grasses (*Ustilago striaeformis*) in the Pacific Northwest. Phytopath. **30**: 93–118. 1940.
4. KREITLOW, K. W. *Ustilago striaeformis*. I. Germination of chlamydospores and culture of forma *agrostidis* on artificial media. Phytopath. **33**: 707–712. 1943.
5. LIRO, J. I. Die Ustilagineen Finnlands Ann. Acad. Sci. Fenn., Ser. A **17**: 1–636. 1924. Cited George W. Fischer. Phytopath. **30**: 93–118. 1940.
6. PAMMEL, L. H. Notes on some fungi common during the season of 1892, at Ames, Iowa. Agr. Sci. **7**: 20–27. 1893.

# NEW AND STANDARD SEED TREATMENTS IN THE CONTROL OF CERTAIN SEED-BORNE DISEASES OF WHEAT, OATS, AND BARLEY[1]

F. J. Greaney and H. A. H. Wallace

(Accepted for publication April 12, 1943)

During the 1942 season, cooperative tests were conducted at several stations in Canada to determine the value of certain new and standard fungicidal seed-treatment materials for the control of smut (*Ustilago avenae* (Pers.) Jens. and *U. levis* (Kell. and Sw.) Magn.) in oats, covered smut (*Ustilago hordei* (Pers.) Lagerh.) in barley, seedling blight (*Helminthosporium sativum* P. K. and B.) in wheat and barley, and leaf blotch (*Helminthosporium avenae* Eidam) in oats.

## MATERIALS AND METHODS

The following dry disinfectants were used: Ceresan, 5 per cent ethyl mercuric phosphate (Bayer-Semesan Co.); "Half Ounce" Leytosan, 7.2 per cent phenyl mercury (F. W. Berk & Co., Ltd., London, England); Spergon, 99 per cent tetrachloro parabenzoquinone (U. S. Rubber Co.); N.G. 3399/9 K, an unnamed dust (Canadian Industries Ltd.); Thiosan (DuBay 1205–FF), 50 per cent tetra-methyl thiuramdisulphide (Bayer-Semesan Co.); Chemical No. 601 and Chemical No. 604 (U. S. Rubber Co.). Ceresan and Leytosan were applied at the rate of ½ oz. per bu. of grain, Spergon and N.G. 3399/9 K dust at 3 oz. per bu., and Thiosan, Chemical No. 601, and Chemical No. 604 at 2 oz. per bu.

In addition to the dry disinfectants mentioned above, the following materials were used as wet treatments: Formaldehyde, Ceresan, and Bordeaux mixture. Formaldehyde was used at the standard rate of one part of Formalin (40 per cent formaldehyde to 320 parts of water). The Ceresan solution consisted of 1 part of Ceresan to 800 parts of water, by weight. The grain was immersed in the formaldehyde and Ceresan solutions for 3 minutes. After treatment it was covered for 4 hours, then dried and placed in small paper envelopes ready for seeding. The Bordeaux mixture used was prepared by dissolving 20 g. of powdered $CuSO_4$ and 20 g. of $Ca(OH)_2$ in 1000 cc. of water. The seed was immersed in this solution for 15 minutes, dried, and packaged. All seed treatments were made from 15 to 30 days prior to seeding time.

Severely naturally infected seed from the crops of 1941 was used in all experiments. The smut tests were made with Anthony oats and Trebi barley. The oat seed carried a smut spore load equivalent to one part of

[1] Experiments carried out in cooperation with the Officers in Charge of the Dominion Laboratories of Plant Pathology at Edmonton, Alta., Saskatoon, Sask., St. Catharines, Ont., Ottawa, Ont., Fredericton, N. B., Kentville, N. S., and Charlottetown, P. E. I., and with Prof. J. G. Coulson, Macdonald College, Quebec. Contribution No. 736, Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

spores (mostly *Ustilago avenae*) to 16,000 parts of seed, by weight, and the barley a spore load of 1:3,500. Seed lots of Charlottetown 80 barley and Reward wheat with 86 per cent and 52 per cent of the kernels, respectively, internally infected with *Helminthosporium sativum*, were used in the seedling-blight tests. Erban oats was used in the oat-leaf-blotch experiments. An agar-plate test of this seed showed that 38 per cent of the kernels was internally infected with *Helminthosporium avenae*. The treated seed, along with similarly diseased nontreated seed of Anthony oats, Trebi barley, Charlottetown 80 barley, and Erban oats, was planted at Winnipeg, Manitoba, in four separate experiments at the rate of 200 seeds per 8-foot row. The system of replication used in each experiment consisted of 4 randomized blocks of 8 rows each. Similar plantings were made at 8 other stations in Western Canada, and at 7 stations in Eastern Canada. The results of these experiments are summarized in tables 1 and 2.

In addition to the above-mentioned cooperative tests, greenhouse and field experiments were made at Winnipeg to determine the effect of certain standard and new fungicidal seed treatment materials, including Spergon and Thiosan, on the control of seedling blight in wheat and barley and of leaf blotch in oats, and on plant emergence and yield. The field experiments were repeated at Morden, Manitoba. In the greenhouse tests, 100 seeds of each kind of treated and untreated seed of Reward wheat, Charlottetown 80 barley, and Erban oats were planted in rows in large beds of non-sterile soil, maintained in proper condition, and in which the amount of soil-borne infection was negligible. Data on emergence and disease control were obtained 15 days after the seed had been sown. The greenhouse tests were run at two different times. Field experiments, similar to those carried out in the cooperative tests, were made at Winnipeg and Morden, Man. In these tests, treated and untreated diseased seed of Reward wheat as well as Charlottetown 80 barley and Erban oats was planted in separate experiments. The materials and rates of application used are given in tables 3 and 4.

Three separate experiments were made at Winnipeg and Morden to determine the effect of different seed treatments on the yield of diseased seed of Reward wheat, Charlottetown 80 barley, and Erban oats. The treated seed, along with untreated seed of each variety, was planted in rod rows at the rate of from 400 to 500 seeds per row, depending upon the variety, replicated 6 times for each fungicide. Thus, each yield test consisted of 6 randomized blocks of 10 rod rows each. Yields were finally computed on the basis of bushels per acre. The results of the yield tests are presented in table 4.

Data on emergence in all field experiments were obtained about 30 days after planting; those for the greenhouse tests, when the seedlings were 15 days old. The disease data of the smut experiments (Table 1) were recorded at or near harvest time. The total number of heads and number of smutted heads per 8-foot row were counted; and the percentage of smutted heads

per row was computed. The method of recording the disease data in the cooperative barley seedling blight and oat leaf blotch tests (Table 2), and in the special wheat and barley seedling blight and oat blotch experiments (Tables 3 and 4), was as follows: Immediately after the emergence data had been recorded, the young plants were lifted from the soil, cleaned, and examined individually for presence of basal or leaf lesions caused by seed-borne fungi, and the percentage of diseased plants per row was determined.

The emergence, disease, and yield data of each field experiment, with the exception of the disease data of the smut experiments, were treated statistically by the analysis of variance method.

## EXPERIMENTAL RESULTS

The results of the oat- and barley-smut experiments are given in table 1. These data show that Ceresan and Leytosan gave perfect control of smut in oats, and almost perfect control of covered smut of barley, while the Ceresan-dip and formalin-dip treatments were only slightly less effective. Spergon and Bordeaux mixture gave only partial smut control. N.G. 3399/9 K dust was quite ineffective in controlling the smuts of oats and barley. The formalin dip treatment reduced emergence in oats, and Ceresan dip, in barley.

TABLE 1.—*Effect of seed treatment with different fungicides on seedling emergence, and on the occurrence of smut in oats, and covered smut in barley. Results of cooperative cereal seed treatment experiments at stations in eastern and western Canada in 1942*

| Fungicide | Oz. per bu. | Oat smut[b] (*U. avenae* and *U. levis*) | | | | Barley smut[c] (*Ustilago hordei*) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean percentage emergence (Emer.) and mean percentage heads smutted (Smut) | | | | | | | |
| | | Eastern, 7 stations | | Western, 9 stations | | Eastern, 6 stations | | Western, 9 stations | |
| | | Emer. | Smut | Emer. | Smut | Emer. | Smut | Emer. | Smut |
| Ceresan | ½ | 78 | 0.0 | 84 | 0.0 | 84 | 0.1 | 87 | 0.01 |
| Leytosan | ½ | 77 | 0.0 | 83 | 0.0 | 82 | 0.2 | 87 | 0.02 |
| Spergon | 3 | 80 | 2.6 | 81 | 0.9 | 84 | 0.9 | 88 | 0.6 |
| N.G. 3399/9 K | 2 | 77 | 3.8 | 77 | 1.9 | 81 | 1.1 | 86 | 1.0 |
| Ceresan dip | ..... | 78 | 0.01 | 84 | 0.2 | 70 | 0.01 | 75 | 0.02 |
| Formalin dip | ..... | 71 | 0.01 | 74 | 0.01 | 82 | 0.9 | 89 | 0.4 |
| Bordeaux mixture | ..... | 76 | 1.4 | 79 | 0.8 | 80 | 1.1 | 83 | 0.9 |
| None | ..... | 78 | 7.1 | 78 | 2.7 | 85 | 4.0 | 87 | 4.1 |
| Necessary difference, 5% level | ..... | 4.3 | [a] | 4.0 | [a] | 4.1 | [a] | 3.7 | [a] |

[a] Smut data not analyzed statistically.
[b] Variety = Anthony. Smut spore load on seed = 1: 16,000.
[c] Variety = Trebi. Smut spore load on seed = 1: 3,500.

The amount of smut observed in the untreated oats at the different stations ranged from 1.3 to 11.2 per cent, with an average of 7.1 per cent for 7 stations in Eastern Canada and 2.7 per cent for 9 stations in Western

Canada. Smut in the untreated barley ranged from 1.1 to 16.4 per cent, and averaged only 4.0 per cent for the 15 stations in Eastern and Western Canada. Obviously, the fungicides were not subjected to a very severe test in 1942. However, owing to the fact that exceedingly uniform smut control results were obtained with each fungicide at 15 widely separated stations, and also to the fact that almost perfect control of smut was obtained at all stations with the organic mercury compounds, Ceresan and Leytosan, it is considered that the results of the 1942 tests give a fairly good indication of the possible effectiveness of the fungicides tested in controlling covered smut of barley and smut in oats.

TABLE 2.—*Effect of seed treatment with different fungicides on seedling emergence, and on the occurrence of seedling blight (Helminthosporium sativum) in barley, and leaf blotch (Helminthosporium avenae) in oats. Results of cooperative cereal seed treatment experiments at stations in eastern and western Canada in 1942*

| Fungicide | Oz. per bu. | Seedling blight of barley[a] (*Helminthosporium sativum*) | | | | Leaf blotch of oats[b] (*Helminthosporium avenae*) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean percentage emergence (Emer.) and mean percentage seedlings diseased (Dis.) | | | | | | | |
| | | Eastern, 5 stations | | Western, 9 stations | | Eastern, 6 stations | | Western, 6 stations | |
| | | Emer. | Dis. | Emer. | Dis. | Emer. | Dis. | Emer. | Dis. |
| Ceresan | ½ | 84 | 3.5 | 89 | 11.6 | 78 | 16.4 | 90 | 3.0 |
| Leytosan | ½ | 84 | 2.7 | 90 | 11.3 | 78 | 23.6 | 89 | 4.3 |
| Spergon | 3 | 62 | 20.5 | 66 | 74.6 | 74 | 42.4 | 86 | 20.5 |
| N.G. 3399/9 K | 2 | 70 | 26.2 | 75 | 77.9 | 78 | 33.9 | 90 | 26.2 |
| Ceresan dip | ..... | 82 | 3.0 | 89 | 10.6 | 74 | 23.8 | 84 | 4.3 |
| Formalin dip | ..... | 77 | 20.0 | 80 | 67.6 | 76 | 33.6 | 85 | 20.0 |
| Bordeaux mixture | ..... | 63 | 25.5 | 68 | 82.2 | 76 | 43.3 | 83 | 25.5 |
| None | ..... | 63 | 25.9 | 70 | 78.9 | 75 | 39.7 | 80 | 25.9 |
| Necessary difference, 5% level | ..... | 4.5 | 13.5 | 4.1 | 6.6 | [c] | 10.1 | 4.6 | 11.2 |

[a] Variety = Charlottetown 80. Percentage of seeds infected = 86 per cent.
[b] Variety = Erban. Percentage of seeds infected = 38 per cent.
[c] Differences between fungicides insignificant statistically.

The results of greenhouse and field experiments designed to determine the effect of different seed treatments on the control of seedling blight (*Helminthosporium sativum*) in wheat and barley, and leaf blotch (*H. avenae*) in oats, and on plant emergence and yield, are given in tables 2, 3, and 4. From these data it is evident that, on the whole, the organic mercury seed-treatment materials, Ceresan, Leytosan, and Ceresan dip, were highly effective in controlling seed-borne diseases of cereals caused by species of *Helminthosporium*, whereas Spergon and Thiosan were quite ineffective. Although Chemical No. 604 gave fairly effective control of leaf blotch of oats and partial control of seedling blight in wheat, it was ineffective in controlling barley seedling blight (Tables 3 and 4). Treatment of severely naturally infected seed of wheat, oats, and barley with Formalin dip, Chem-

ical No. 601, Bordeaux mixture, and N.G. 3399/9 K dust, did not reduce the incidence of seedling blight in either wheat or barley, or of leaf blotch in oats. The results obtained in beds of ordinary soil in the greenhouse agreed very closely with those obtained in the field.

TABLE 3.—*Effect of seed treatment with different fungicides on the control of seedling blight in wheat and barley and leaf blotch of oats, and on seedling emergence. Results of greenhouse soil-bed tests.* (*Data are means of 2 trials*)

| Fungicide | Oz. per bu. | Seedling blight of wheat[a] (*H. sativum*) | | Seedling blight of barley[b] (*H. sativum*) | | Leaf blotch of oats[c] (*H. avenae*) | |
|---|---|---|---|---|---|---|---|
| | | Percentage seedling emergence | Percentage seedlings diseased | Percentage seedling emergence | Percentage seedlings diseased | Percentage seedling emergence | Percentage seedlings diseased |
| Ceresan | ½ | 98 | 0 | 98 | 0 | 95 | 0 |
| Leytosan | ½ | 96 | 0 | 99 | 1 | 96 | 0 |
| Spergon | 3 | 92 | 32 | 89 | 66 | 94 | 18 |
| Chemical #601 | 2 | 75 | 49 | 90 | 44 | 94 | 16 |
| Chemical #604 | 2 | 94 | 18 | 96 | 36 | 95 | 10 |
| Thiosan | 2 | 97 | 21 | 90 | 33 | 92 | 9 |
| N.G. 3399/9 K | 3 | 78 | 25 | 89 | 50 | 92 | 11 |
| Ceresan dip | ..... | 98 | 0 | 96 | 0 | 94 | 0 |
| Bordeaux mixture | ..... | 87 | 48 | 86 | 56 | 95 | 18 |
| None | ..... | 70 | 46 | 86 | 50 | 93 | 14 |

[a] Variety = Reward. Percentage of seeds infected = 52 per cent.
[b] Variety = Charlottetown 80. Percentage of seeds infected = 86 per cent.
[c] Variety = Erban. Percentage of seeds infected = 38 per cent.

The field results in table 4 show that Ceresan, Leytosan, Spergon, Thiosan, N.G. 3399/9 K dust, and Chemical No. 604, were consistently beneficial to emergence, while Bordeaux mixture, and, in general, Chemical No. 601, were of little benefit in this respect. As might be expected from the disease control results, highly significant increases in yield were obtained over nontreated seed when wheat seed severely infected with *Helminthosporium sativum* was treated with Ceresan or Leytosan. Most of the other fungicides also increased yield significantly. In general, wheat yield was increased in direct proportion to the amount of seedling blight control. Unfortunately, individual plot yields of the oat and barley experiments varied widely in 1942, and hence the yield differences observed between seed treatments in these tests were, for the most part, statistically insignificant.

The results presented in tables 1, 2, 3, and 4 show the marked effectiveness of the organic mercury seed disinfectants, Ceresan and Leytosan, in controlling certain destructive seed-borne diseases of wheat, oats, and barley. In comparison with these standard fungicides, the new materials, Spergon, Thiosan, Chemical No. 601, and Chemical No. 604, were quite ineffective. Spergon is on the market as a vegetable seed protectant and Thiosan as a fungicide for turf. Chemical No. 601 and Chemical No. 604 are still in the experimental stage. In 1942, Spergon and Thiosan were quite unsatis-

TABLE 4.—*Effect of seed treatment with different fungicides on the occurrence of seedling blight in wheat and barley, and of leaf blotch in oats, and on seedling emergence and yield. Results of field experiments at Winnipeg and Morden, Manitoba, in 1942*

| Fungicide | Oz. per bu. | Winnipeg | | | Morden | | |
|---|---|---|---|---|---|---|---|
| | | Seedling blight of wheat[b] (*Helminthosporium sativum*) | | | | | |
| | | Percentage seedling emergence | Percentage seedlings diseased | Yield (bu. per ac.) | Percentage seedling emergence | Percentage seedlings diseased | Yield (bu. per ac.) |
| Ceresan | ½ | 74 | 18.5 | 53.6 | 92 | 15.1 | 42.9 |
| Leytosan | ½ | 75 | 17.4 | 54.1 | 94 | 15.6 | 41.2 |
| Spergon | 3 | 71 | 48.6 | 41.6 | 68 | 49.4 | 32.6 |
| Chemical #601 | 2 | 49 | 60.5 | 34.2 | 41 | 59.9 | 23.8 |
| Chemical #604 | 2 | 79 | 40.7 | 50.1 | 80 | 48.3 | 37.7 |
| Thiosan | 2 | 74 | 50.8 | 48.3 | 77 | 51.2 | 37.1 |
| N.G. 3399/9 K | 3 | 62 | 55.9 | 44.2 | 63 | 60.8 | 30.3 |
| Ceresan dip | ..... | 82 | 19.1 | 51.8 | 92 | 22.8 | 36.2 |
| Bordeaux mixture | ..... | 56 | 57.4 | 37.8 | 44 | 58.8 | 27.2 |
| None | ..... | 40 | 60.4 | 28.2 | 23 | 56.4 | 19.4 |
| Necessary difference, 5% level | ..... | 10.5 | 13.2 | 2.8 | 7.4 | 8.8 | 5.1 |
| | | Seedling blight of barley[c] (*Helminthosporium sativum*) | | | | | |
| Ceresan | ½ | 91 | 18.3 | 39.4 | 91 | 18.6 | 53.8 |
| Leytosan | ½ | 87 | 22.7 | 37.1 | 92 | 23.3 | 54.4 |
| Spergon | 3 | 72 | 58.4 | 38.6 | 73 | 73.8 | 51.5 |
| Chemical #601 | 2 | 78 | 57.3 | 41.7 | 74 | 92.9 | 49.6 |
| Chemical #604 | 2 | 76 | 58.9 | 38.1 | 80 | 77.8 | 53.7 |
| Thiosan | 2 | 77 | 48.9 | 38.1 | 78 | 86.6 | 57.7 |
| N.G. 3399/9 K | 3 | 79 | 59.1 | 37.0 | 74 | 88.8 | 57.3 |
| Ceresan dip | ..... | 85 | 16.2 | 35.7 | 88 | 18.8 | 48.1 |
| Bordeaux mixture | ..... | 69 | 66.0 | 35.6 | 66 | 90.7 | 42.6 |
| None | ..... | 67 | 62.3 | 31.3 | 72 | 91.5 | 45.7 |
| Necessary difference, 5% level | ..... | 8.6 | 6.8 | [a] | 5.8 | 7.2 | 9.1 |
| | | Leaf blotch of oats[d] (*Helminthosporium avenae*) | | | | | |
| Ceresan | ½ | 86 | 2.6 | 58.0 | 92 | 2.4 | 95.5 |
| Leytosan | ½ | 86 | 1.9 | 62.6 | 93 | 2.1 | 93.8 |
| Spergon | 3 | 80 | 57.0 | 62.5 | 80 | 42.9 | 84.6 |
| Chemical #601 | 2 | 81 | 55.0 | 53.9 | 82 | 48.8 | 91.4 |
| Chemical #604 | 2 | 80 | 33.9 | 59.5 | 86 | 23.2 | 97.4 |
| Thiosan | 2 | 82 | 44.9 | 60.7 | 89 | 33.0 | 88.0 |
| N.G. 3399/9 K | 3 | 73 | 31.0 | 60.4 | 88 | 33.5 | 90.0 |
| Ceresan dip | ..... | 79 | 1.9 | 65.1 | 91 | 2.4 | 98.5 |
| Bordeaux mixture | ..... | 74 | 49.3 | 60.4 | 86 | 42.8 | 82.7 |
| None | ..... | 75 | 57.2 | 61.4 | 82 | 55.5 | 82.8 |
| Necessary difference, 5% level | ..... | [a] | 9.8 | [a] | 4.1 | 8.6 | [a] |

[a] Differences between fungicides insignificant statistically.
[b] Variety = Reward wheat. Percentage of seeds infected = 52 per cent.
[c] Variety = Charlottetown 80. Percentage of seeds infected = 86 per cent.
[d] Variety = Erban. Percentage of seeds infected = 38 per cent.

factory for the control of seedling blight of wheat and barley, and leaf blotch of oats, but further tests with these new non-metallic fungicides against these and other destructive seed-borne diseases of cereals would be desirable.

## SUMMARY

Field experiments were made in 1942 to determine the effect of certain standard and new fungicidal seed treatment materials on the control of smut (*Ustilago avenae* and *U. levis*) in oats, covered smut (*Ustilago hordei*) in barley, seedling blight (*Helminthosporium sativum*) in wheat and barley, and leaf blotch (*Helminthosporium avenae*) in oats. Severely naturally infected seed was used in all experiments.

The organic mercury seed disinfectants, Ceresan, Leytosan, and Ceresan dip, gave excellent control of smut in oats, covered smut of barley, seedling blight of wheat and barley, and leaf blotch of oats. Formalin dip controlled smut effectively, but was quite ineffective in controlling barley seedling blight and oat leaf blotch. In 1942, the new synthetic organic chemicals, Spergon and Thiosan, gave little promise as cereal seed disinfectants.

DOMINION LABORATORY OF PLANT PATHOLOGY,
WINNIPEG, MANITOBA.

# A NEW METHOD FOR COMPUTING SUGAR BEET LEAF AREA[1]

WILLIAM DOWELL BATEN[2] AND J. H. MUNCIE[3]

(Accepted for publication March 29, 1943)

Many agricultural research workers have interested themselves in the past few years with finding the areas of leaves of various plants. Gustafson and Stoldt (5) studied the relation between leaf area and fruit size in tomatoes. Haller and Magness (6) investigated the association of leaf area and the growth and composition of apples, and Schuster (16) carried out experiments for determining the influence of leaf area and shoot growth in relation to size and filling of filberts. Davis (2) developed a formula for finding areas of bean leaves, and Porter[4] found a parabolic formula for areas of tomato leaves. Magness and Overley (10, 11) investigated the effect of leaf area upon size and quality of apples and pears, and Overholser and Claypool (14) carried out research on the relation of leaf area per peach to physical properties and chemical composition. Swanson (17) studied the influence of leaf area of sorghum versus yield. Mitchell (13), Frear (3), Gerdel and Salter (4), Kramer (9), Withrow (18), Hibbard, Grigsby and Keck (8) developed photo-electric devices for the measurement of leaf areas and Marshall (12) constructed an apparatus for obtaining the areas of raspberry leaves. Baten constructed a nomogram for finding the area of bean leaves (1).

There are several experiments now being carried out at Michigan State College in which areas of leaves form important parts of the investigations; these pertain to counts of European red mites per leaf area of Delicious apple tree leaves and effectiveness of sprays; effects of sprays in relation to transpiration of sugar-beet leaves and honeysuckle leaves; leaf area versus yield of field beans; etc.

In experiments designed to determine the effect of spray or dust materials upon transpiration rate of plants, it is necessary to employ an accurate and rapid method of finding the leaf area. The standard method of finding leaf area is by means of the planimeter. Although this method is accurate, it involves a great amount of tedious, painstaking effort and consumption of valuable time.

In recent experiments designed to determine the effect of copper sprays and dusts upon the transpiration rate of sugar beet leaves in the greenhouse it became highly desirable to employ some method other than planimeter readings for determining leaf area. Sugar-beet leaves of varying ages were

[1] Published by permission of the Director, Michigan Agricultural Experiment Station as Journal Article number 636 (new series).

[2] Research Assoc. in Statistics, Mich. Agr. Exp. Station.

[3] Extension Specialist and Res. Assoc. in Plant Pathology, Mich. State College.

[4] Porter, A. M. The influence of defoliation and fruit thinning on the growth of tomatoes. Unpublished thesis, Michigan State College, 1932.

cut from the plant, and dried between blotters in a plant press. The ruffled leaf margins were smoothed as much as possible before the leaves were completely dried. After drying, planimeter readings were made of the area of 200 leaves. These were then used in checking against the data obtained by using a new method derived by the writers.

Areas of the leaves, obtained by the planimeter, were plotted against the widths, the lengths, widths and lengths, and also against the products of the widths and lengths. Straight lines were found by the method of least squares, for predicting leaf areas from these respective measurements. The standard errors of estimate, which indicate how accurate predictions are,

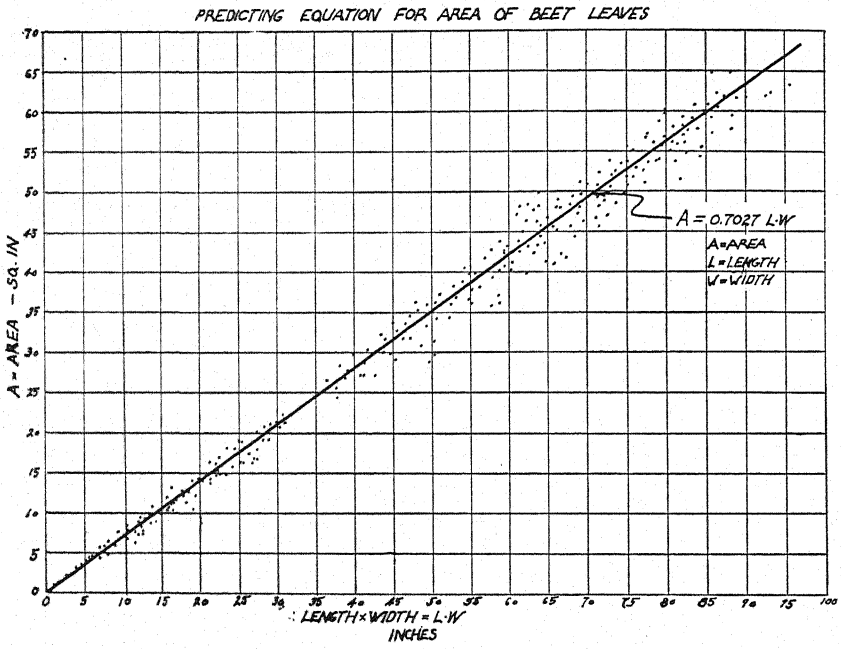



FIG. 1.

were found in each case, together with correlation coefficients. The smallest standard error of estimate was found where the product of width and length was used for predicting leaf area. The predicting equations pertaining to leaf area and product of length and width are as follows:

Small leaves—1941: $A = 0.6974P$,
Large leaves—1942: $A = 0.7035P$,
All leaves: $A = 0.7027P$,

where A represents leaf area and P represents the product of width and length. These equations without a constant term were about as reliable as the equations with such a term; these equations with a constant term are not given. Since the equation for predicting areas for small leaves differs very little from that for predicting areas for large leaves, all of the data were combined for obtaining one predicting equation. This equation is

$$A = 0.7027P;$$

and it was used in constructing the nomogram. Figure 1 shows the scatter diagram about this straight line.

The nomogram was obtained by using the linear relation between the logarithms of the area, 0.7027L and W as

$$\text{Log } A = \text{Log } 0.7027L + \text{Log } W.$$

The scales on each of the axes, shown in figure 2, were obtained as logarithmic scales for the chosen interval of 12 inches for the heights of the axes of the measurements pertaining to width and length measurements. Methods for constructing nomograms are given in any text on nomography (7, 15).

The area of a sugar-beet leaf with width and length equal, respectively, to 3.4 in. and 4.5 in. is found by laying a straight edge on the width axis (Nomogram, Fig. 2) on 3.4 and on the length axis on 4.5 and then reading the area of the leaf on the area axis; this is found to be 10.8 sq. in. The area of a sugar-beet leaf with width and length respectively equal to 7.6 in. and 9.2 inches is found by laying the straight edge on the width axis on 7.6 and the length axis on 9.2 and then reading the area on the area axis, where the straight edge crosses this axis; this is 49.1 sq. in. By using the nomogram, one can find the areas of the leaves in the field or in the greenhouse without removing them from the plants and without any computations. One person can measure the width and length of the leaf on the plant, while a second person reads from the nomogram, for these measurements, the area of the leaf. It is necessary only to record one value, the area of the leaf. In this way 2 people can determine the areas of many leaves in a relatively short time. By this method the plants are not mutilated.

The nomogram is of little value unless one is going to measure many leaves or is going to carry on the study with the same kind of leaves at least 2 years, or when the equation for finding leaf area in terms of length and width measurements does not hold from season to season. However, if the investigation is going to extend over several years, as many do, and the equation obtained from measurements the first years is suitable for the measurements of the same kind of leaves the succeeding years, as often happens, then the nomogram constructed from the first-year measurements will be beneficial in all future work. Davis (2) has used an equation for the area of bean leaves several seasons; in his case the nomogram will greatly reduce the amount of time and labor devoted to finding leaf areas. Many people use the information gathered the first year or for the first four or five hundred leaves for determining the areas of all future leaves; in these cases it would pay to construct a nomogram from the first set of data and use it for future work.

This article was written for the purpose of calling attention to this very handy method of finding the area of leaves. Similar methods might be used for other relations. The nomogram for obtaining areas of beet leaves will not be applicable for other kinds of leaves.

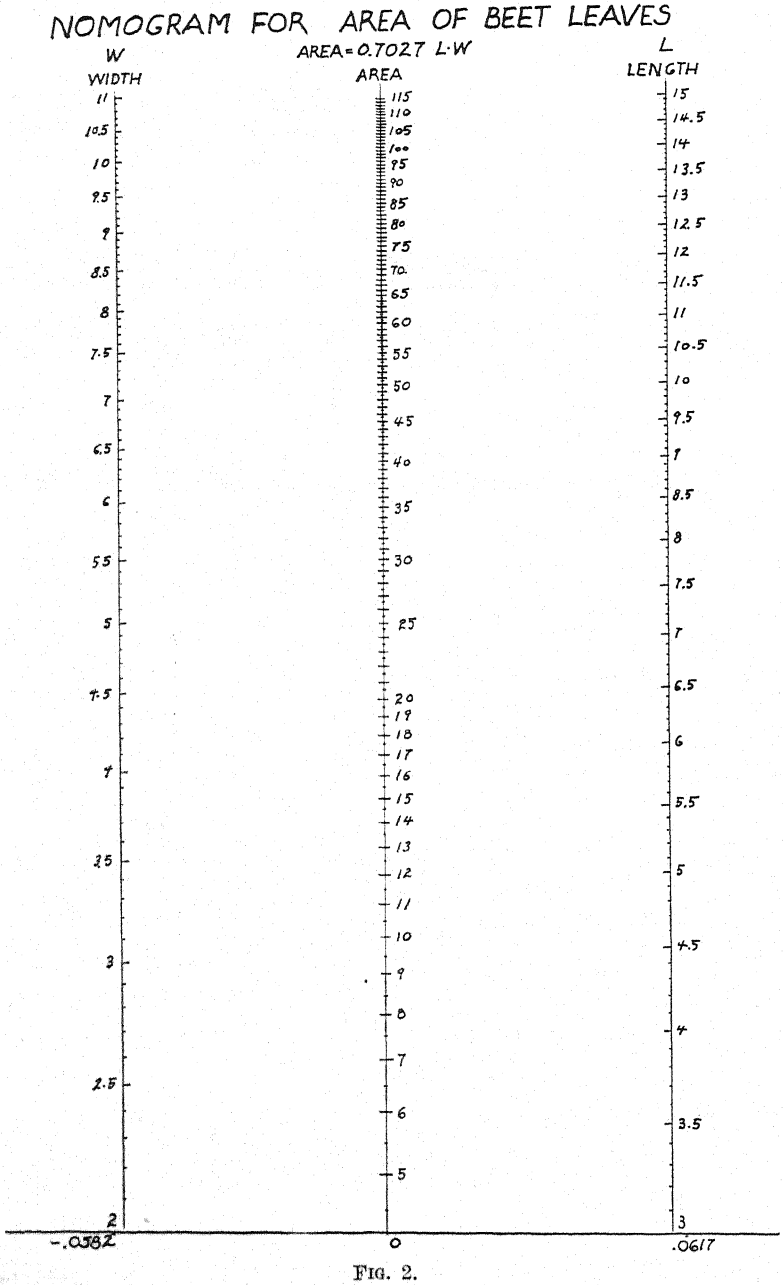

NOMOGRAM FOR AREA OF BEET LEAVES
AREA = 0.7027 L·W
W
WIDTH
AREA
L
LENGTH
-.0382
0
.0617

FIG. 2.

SUMMARY

A method has been devised for rapid determination of areas of sugar-beet leaves. For this purpose a nomogram was obtained by using the linear relation between the logarithms of the area, the length and the width of the leaves.

LITERATURE CITED

1. BATEN, WILLIAM DOWELL. A nomogram for finding the areas of bean leaves. Jour. Amer. Soc. Agron. 34. 1942.
2. DAVIS, J. F. The relationship between leaf area and yield of the field bean with a statistical study of methods for determining leaf area. Jour. Amer. Soc. Agron. 32: 323–329. 1940.
3. FREAR, D. E. H. Photoelectric apparatus for measuring leaf areas. Plant Phys. 10: 569–574. 1935.
4. GERDEL, R. W., and R. M. SALTER. Measurement of leaf area using the photoelectric cell. Jour. Amer. Soc. Agron. 20: 635–642. 1928.
5. GUSTAFSON, F. G., and ELNORE STOLDT. Some relations between leaf area and fruit size in tomatoes. Plant Phys. 11: 445–451. 1936.
6. HALLER, M. H., and J. R. MAGNESS. The relation of leaf area to the growth and composition of apples. Proc. Amer. Soc. Hort. Sci. 22: 189–196. 1925.
7. JOHNSTON, L. S. The elements of nomography. (Mimeographed) 83 pp. Detroit. University of Detroit. 1936.
8. HIBBARD, R. P., B. H. GRIGSBY, and W. G. KECK. A low light intensity photoelectric device for the measurement of leaf areas. Papers Mich. Acad. Science, Arts, and Letters. 23: 141–147. 1937.
9. KRAMER, P. J. An improved photoelectric apparatus for measuring leaf areas. Amer. Jour. Bot. 24: 375–376. 1937.
10. MAGNESS, J. R. Relation of leaf area to size and quality in apples. Proc. Amer. Soc. Hort. Sci. 25: 285–288. 1928.
11. ————————, and F. L. OVERLEY. Relation of leaf area to size and quality of apples and pears. Proc. Amer. Soc. Hort. Sci. 26: 160–162. 1929.
12. MARSHALL, R. E. An apparatus for the ready determination of areas of compound leaves. Jour. Agr. Res. [U.S.] 47: 437–439. 1933.
13. MITCHELL, J. W. Measurement of the area of attached and detached leaves. Science 83: 334–336. 1936.
14. OVERHOLSER, E. L., and L. L. CLAYPOOL. The relation of leaf areas per peach to physical properties and chemical composition. Proc. Amer. Soc. Hort. Sci. 28: 15–17. 1931.
15. PLANT, L. C., and T. R. RUNNING. First year college mathematics. American Book Company. 424 pp. (New York). 1939.
16. SCHUSTER, C. E. Leaf area and short growth in relation to size and filling of filberts. Proc. Amer. Soc. Hort. Sci. 30: 392–395. 1933.
17. SWANSON, A. F. Relation of leaf area to grain yield of sorghum. Jour. Amer. Soc. Agron. 33: 18–22. 1931.
18. WITHROW, R. B. A photoelectric device for the rapid measurement of leaf area. Jour. Agr. Res. [U.S.] 50: 637–643. 1935.

# DISTRIBUTION OF BACTERIAL WILT (BACTERIUM SOLANACEARUM) IN SUCCESSIVE CROPS OF TOBACCO GROWN ON THE SAME FIELDS[1]

T. E. SMITH[2]

(Accepted for publication March 30, 1943)

## INTRODUCTION

Bacterial wilt occurs in spots in many tobacco fields. In some areas 50 to 100 per cent of the plants may be affected, while in other areas of the same field one per cent or less may be diseased (Fig. 1).

Whether or not these patterns of distribution remain constant or change from season to season was of importance in studying control measures. A marked increase in wilt severity in an area of the field where it was present only in small amount or absent on the previous tobacco crop would indicate that the pathogen had been spread by surface water or other means. Therefore, cultural practices that reduced rapidity of spread would be of value in controlling the disease. Information was not available on the stability of the pattern of occurrence and to study this problem maps were made of wilt distribution during two or more successive crops of tobacco.

## METHODS

In the fields selected for study wilt had been present in varying amount for perhaps 20 to 30 years. According to the farmers each field had been wholly planted to tobacco or other crops in 1936 and 1937. The survey was undertaken in 1938 by dividing the fields into plots of $50 \times 50$ ft., an area sufficient for approximately 250 tobacco plants. Wilt counts were made during the first few days of August. Concurrently, the elevation of each plot above the lowest point in the field was determined with a transit. Plots were laid out and data taken on a total area of 11.9 acres in 7 fields. All plots, except 3, contained some wilted tobacco plants in 1938. Four of the fields were planted to tobacco in 1940 or 1942, depending on the length of rotation used, and at that time the plots on these fields were relocated for additional wilt counts. Early-season incidence was studied on 2 fields in counts made June 10, about 5 weeks after transplanting. Final distribution of the disease was studied in counts made August 1 to 15, on completion of harvesting. The number of plots, average amount of wilt in 1938, rotation used, and average amount of wilt on the succeeding crop of tobacco are

[1] Cooperative investigations of the Division of Tobacco Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, the N. C. Department of Agriculture, and the N. C. Agricultural Experiment Station. Published with the approval of the Director as paper No. 161 of the Journal series.

[2] The writer expresses appreciation to R. K. Godfrey and J. F. Gilmore for assistance in the field work and to the Department of Experimental-Statistics of the Experiment Station for assistance in the statistical computations.

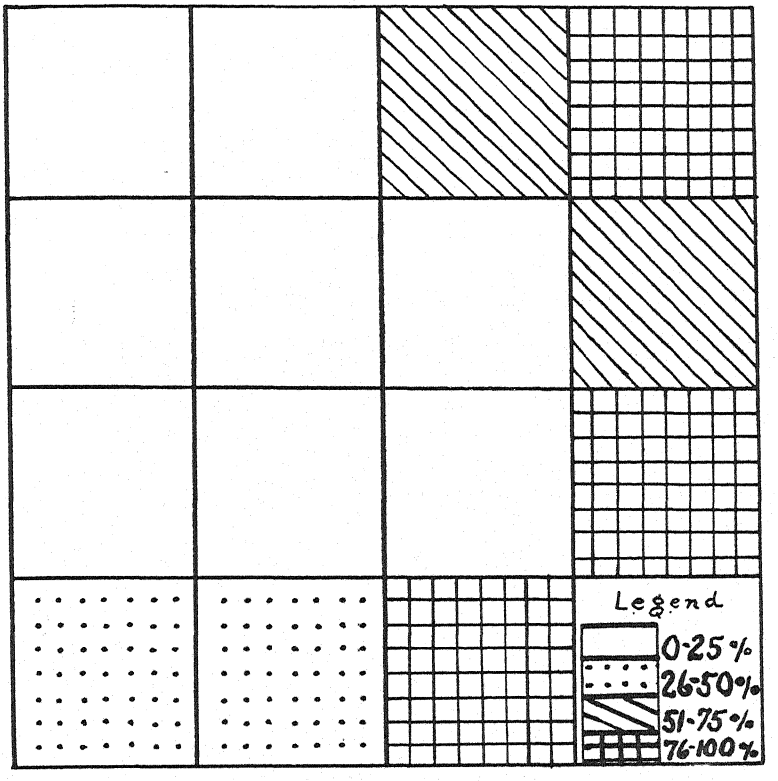



FIG. 1. Distribution of bacterial wilt in a tobacco field. Each square represents an area of 50 × 50 feet with the indicated amount of wilt.

TABLE 1.—*Number of plots, average amount of wilt and rotations used in the fields studied*

| Field | Plots | Amount of wilt on tobacco grown in 1938 | Rotation used | Amount of wilt on tobacco grown in 1940 or 1942 |
|---|---|---|---|---|
| | *Number* | *Per cent* | *Year and crop grown* | *Per cent* |
| 1 | 32 | 7 ± 9[a] | 1939, crab grass<br>1940, tobacco<br>1941, corn<br>1942, tobacco | 11 ± 11<br>25 ± 22 |
| 2 | 12 | 42 ± 33 | 1939, lespedeza<br>1940, tobacco | 52 ± 33 |
| 3 | 15 | 38 ± 30 | 1939, corn<br>1940, tobacco | 71 ± 21 |
| 4 | 50 | 63 ± 22 | 1939, wheat and lespedeza<br>1940, lespedeza<br>1941, corn<br>1942, tobacco | 85 ± 12 |
| 5 | 31 | 14 ± 12 | | |
| 6 | 31 | 62 ± 17 | | |
| 7 | 37 | 53 ± 19 | | |

[a] Mean and standard deviation.

shown in table 1. The amount of wilt varied widely between fields and within fields, as shown by these data.

### RELATION OF WILT SEVERITY IN 1938 OR 1940 TO THAT OF SUCCEEDING CROP

The first problem studied was the relation of wilt severity in 1938 to the areas of the field where wilt first occurred in the tobacco crop the next year. Table 2 presents the data on a field of 12 plots that, for convenience, have been arranged on the basis of the amount of wilt in each plot in 1938. Wilt varied among the different plots from 6 to 96 per cent at the end of the season in 1938. In 1940, 5 weeks after the tobacco was set, some wilt was present on nearly all plots, but the highest per cent occurred on the plots with the most wilt in 1938. The amount of wilt at the end of the season in 1940 was closely related to the early-season incidence. Data on another field of 32 plots were similar to those reported in table 2.

TABLE 2.—*Relation of wilt distribution in 1938 to early season incidence and distribution in 1940*

| Plot | Amount of wilt during the stated years | | |
|---|---|---|---|
| | 1938 | 1940 | |
| | At end of season | Five weeks after transplanting | At end of season |
| *Number* | *Per cent* | *Per cent* | *Per cent* |
| 6 | 6 | 2 | 11 |
| 2 | 9 | 2 | 20 |
| 10 | 15 | 2 | 23 |
| 5 | 15 | 2 | 27 |
| 1 | 20 | 0 | 24 |
| 9 | 26 | 2 | 38 |
| 7 | 37 | 7 | 42 |
| 3 | 48 | 9 | 60 |
| 11 | 51 | 10 | 55 |
| 8 | 82 | 10 | 97 |
| 4 | 96 | 16 | 99 |
| 12 | 96 | 26 | 99 |

The relationship between pattern of occurrence of wilt in successive tobacco crops was determined by means of correlation coefficients. In field numbers 1, 2, 3, and 4 (Table 1), data were available on the percentage of wilted plants on each plot in 1938 and in 1940 or 1942, depending on the length of rotation used. The values of "r" between the amount of wilt present in 1938 and on the next tobacco crop were 0.9645**,[3] 0.9307**, 0.8011** and 0.7115**. Field number 1 was planted in tobacco during 1938, 1940, and 1942. The multiple correlation coefficient (R) between percentage of plants wilted in 1938 ($x_1$), percentage of plants wilted in 1940 ($x_2$) and percentage of plants wilted in 1942 (y) was 0.9052**. The over-all correlation "r" for the entire set of data on 141 plots between per-

[3] ** Highly significant.

centage of wilted plants in 1938 or 1940 and percentage of wilted plants in the next tobacco crop was 0.9036**. All of these coefficients are quite high, showing a close relationship between the pattern of wilt distribution on tobacco grown during different seasons.

### RELATION OF ELEVATION TO WILT SEVERITY

The relation between elevation and wilt severity was studied in ten sets of data, using wilt counts made in 1938, 1940 and 1942. Correlation coefficients (r) were computed between elevation above the lowest point in the field and the percentage of wilted tobacco plants. These were 0.3317*,[4] 0.2583, 0.1895, −0.0444, −0.2183, −0.3271, −0.3512*, −0.4438, −0.8845**, −0.9053**. The coefficients were highly variable, ranging from positive to negative with statistically significant values on both sides of zero. However, the negative relationship was found in seven of the ten sets of data. Hence, in most fields wilt was more severe in the lower-lying areas but this relationship was not consistent for all fields.

### DISCUSSION

The factors that might explain the wide differences in amount of wilt in different parts of a field may, for purposes of discussion, be divided into 2 groups. The first group includes factors that change from season to season, such as the chance introduction of inoculum into the field, spread of inoculum within the field from sites where it had overwintered in the soil, biological effects that might change disease severity and others. If wilt distribution were largely dependent on any or all of these factors, the pattern of distribution would change from year to year. No evidence of a major change in wilt distribution was found; therefore, it is doubtful that such seasonal factors governed the amount of wilt in different areas of the fields studied. The second group of factors determining wilt distribution includes the relatively stable characteristics of the soil and physical features of the field that might effect pathogen or host. Several writers, van der Meer[5] and others, have pointed out that bacterial wilt was more severe on soils of high moisture content. This factor was associated with wilt distribution in some fields. For example, one end of field No. 2 bordered a poorly drained woodland, and inspection showed that soil moisture was considerably higher in the plots in that part of the field. The highest percentages of wilted plants also occurred in the plots nearest the poorly drained woodland, indicating a relationship with soil moisture. However, in other fields, with what appeared to be well-drained soil throughout, variations in amount of wilt also occurred, showing that factors other than proximity of the water table played a part in determining wilt distribution. Bacterial wilt is water-borne and it was expected that repeated movement of contaminated

[4] * Significant.

[5] van der Meer, J. H. H. The influence of degree of soil humidity on slime disease caused by *Bacterium solanacearum*. Bull. Deli Proefsta. (Medan, Sumatra) 29, 1929. (In Dutch with English summary, pp. 36 to 49.)

soil by surface water would cause uniformly severe wilt damage in the lower-lying plots. But, as pointed out previously, the correlation between elevation and amount of wilt varied widely on different fields, showing that direction of flow of surface water was not uniformly associated with wilt distribution. The over-all correlation coefficient of 0.9036** between the amount of wilt in the preceding crop and the succeeding crop suggested that wilt severity was mainly determined by the location of the plot in the field. It appeared that bacterial wilt was in equilibrium with the more stable chemical and physical properties of the soil.

The problems involved in control of bacterial wilt by crop rotation were discussed in an earlier publication.[6] It was shown that the pathogen was not starved out by as much as 4 years of bare fallow. It also was shown that elimination of wilt-susceptible weeds from the immune crop did not increase the degree of control obtained from the rotation. These results illustrate the difficulties of controlling the disease by crop rotation. The data reported here are related to control of the disease by methods of sanitation. Bacterial wilt recurs in proportion to the amount present in the previous tobacco crop. Hence, in fields where wilt has been present for several years, cultural practices tending to reduce spread appear to be of negligible value.

## SUMMARY

Bacterial wilt tends to be unevenly distributed in many tobacco fields. Information was lacking as to whether the pattern of occurrence was similar in successive crops.

Data from 141 plots showed a strong positive correlation between the amount of wilt in 1938 or 1940 and the amount of wilt on the same plots when planted in tobacco 2 or 4 years later.

Wilt was usually, but not always, more severe on the low-lying areas of fields.

The similarity of the pattern of occurrence from year to year suggests that the uneven distribution was associated with permanent soil conditions rather than the random spread of inoculum by cultivation or surface water.

TOBACCO EXPERIMENT STATION, OXFORD, NORTH CAROLINA

[6] Smith, T. E. Control of bacterial wilt (*Bacterium solanacearum*) of tobacco as influenced by crop rotation and chemical treatment of the soil. U. S. Dept. Agr. Circular (in press).

# RHIZOCTONIA ROOT CANKER OF ALFALFA (MEDICAGO SATIVA)[1]

OLIVER F. SMITH[2]

(Accepted for publication April 1, 1943)

The Rhizoctonia root canker of alfalfa herein described was first observed by the writer at the U. S. Yuma Field Station, Bard, California, in May, 1940. An examination of alfalfa roots from many experimental plots on the station showed that a large percentage of the plants were affected, and it appeared that affected plants were being damaged considerably. It is difficult to determine how long the disease has been present in that region, but information obtained by correspondence and conversation with S. H. Hastings and E. G. Noble, Division of Irrigation Agriculture, Bureau of Plant Industry, indicates that it has been present for several years. As far as the writer is aware, no previous description of the disease has been published, nor has its cause been determined. Experiments herein reported show that the disease is caused by *Rhizoctonia solani* Kühn (*Corticium solani* (Prill. and Del.) Bourd. and Gal.).

## DESCRIPTION OF THE DISEASE

The disease is characterized by dark, sunken areas, which sometimes have a brownish border (Fig. 1, B, C, D, and E). Diseased areas are usually circular, but in some cases they are oblong and extend part-way around the root. They generally occur where young roots emerge from larger ones, as evidenced by the fact that near the center of each lesion is often found the dead stub of a small root. Lesions often develop inward to the central region of the root, but the latter usually is not completely rotted off. There is very little spread of the disease up or down the root from the lesion.

The malady is seasonal in its development and closely correlated with high temperature. Lesions develop mainly in June, July, August, and September, when soil temperature, at 3 inches below the soil surface, ranges from about 21 to 35° C., whereas, there is practically no disease development during the winter months, when soil temperature, at 3 inches below the soil surface, may be as low as 5 to 10° C. Lesions that develop in summer usually heal-over during the winter, and by mid-winter or early spring there is often only a scar on the root to indicate the location of the diseased area from the previous summer season (Fig. 1, A). Under controlled conditions, the disease has developed abundantly on alfalfa roots grown at a soil temperature of 25–30° C.; whereas it has developed very little, if at all, on roots grown at a soil temperature of 16–18° C.

[1] Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Nevada Agricultural Experiment Station.

[2] Associate Pathologist, Division of Forage Crops and Diseases.

GEOGRAPHIC DISTRIBUTION OF THE DISEASE

The disease is known to occur in southern California and southwestern Arizona, but may be present also in other areas. It has been reported[3] in the Imperial Valley of Imperial County and the Palo Verde Valley of Riverside County, California. Also it occurs in the Yuma Valley, Yuma Mesa, South Gila, North Gila, Welton-Roll, and Parker Indian Reservation Districts of Yuma County, Arizona.

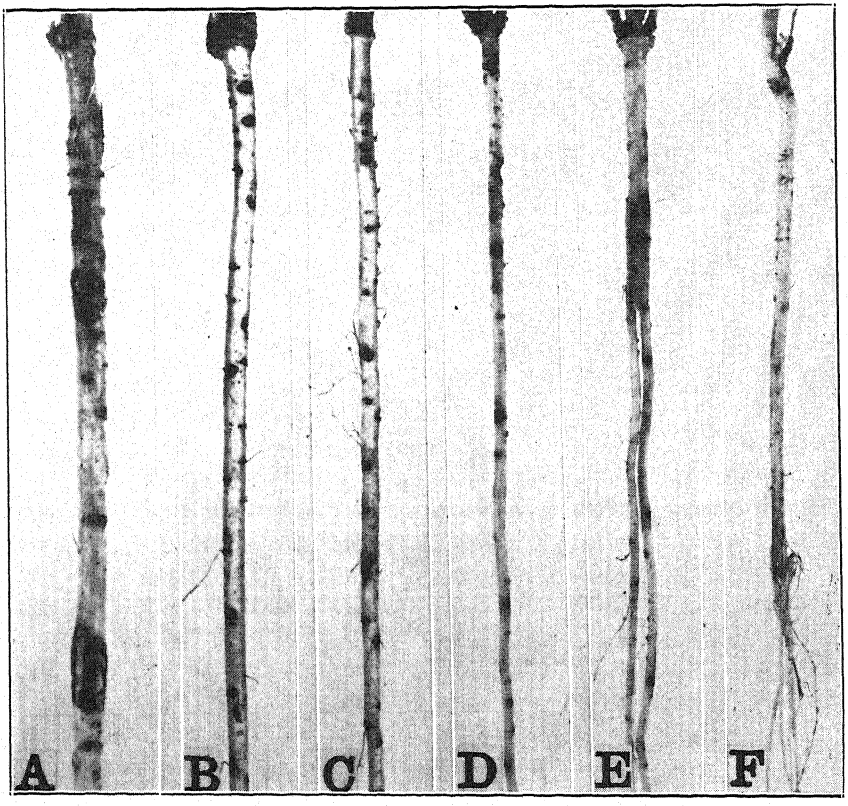



FIG. 1. Symptoms of rhizoctonia root canker of alfalfa. A. Root with scar showing diseased area largely healed over; B and C. Typical lesions on roots as a result of natural infections. D and E. Lesions on roots as a result of inoculation with *Rhizoctonia solani* at a soil temperature of 25–30° C. F. Control plant, not inoculated, but grown at a soil temperature of 25–30° C.

ECONOMIC IMPORTANCE OF THE DISEASE

The economic importance of this disease is not well defined. In regions where it is known to occur alfalfa stands are of short duration, with plant mortality being highest during the summer months, when this disease is most prevalent. It is unlikely, however, that the disease alone is respon-

[3] Unpublished data of a survey made by R. E. Beckett, Division of Irrigation Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, October, 1940.

sible for all the dying of young stands. It appears to be responsible for the death of many small roots, as well as some larger ones, and thus contributes to factors causing plant mortality. It must, therefore, be considered of real economic importance. The relative amount of resultant damage, however, awaits a more thorough knowledge of the entire disease complex in the areas where it occurs.

## CAUSAL ORGANISM

The disease is caused by a fungus identified as *Rhizoctonia solani* Kühn.[4] This fungus has been isolated from many naturally developed lesions on alfalfa roots; and by inoculation experiments with this organism the disease has been reproduced and the organism reisolated.

## PATHOGENICITY TESTS

Pathogenicity tests were made by growing young plants in infested soil. Plants about 3 months old were used, for at this age they bear roots large enough to test the ability of the fungus to cause root lesions. They were transplanted to galvanized-iron cans, 7 inches in diameter and 12 inches deep. In all cases transplants were allowed to grow at a soil temperature of 16–20° C., which is unfavorable for disease development, for at least 4 weeks before the soil temperature was raised to 25–30° C., which is favorable to disease development. The soil was infested with barley grain, previously sterilized, on which the fungus had grown for about 2 weeks. The grain, applied at the rate of about 20 g. (dry weight, prior to soaking and sterilization) to 7000 g. of soil, was added either by mixing with the soil just before transplanting the plants, or the transplants were allowed to grow about a month in the non-infested soil; then 5 holes, about 0.25 inch in diameter and 8 to 10 inches deep, were made in each can of soil, and the inoculum placed in the holes. Both methods gave satisfactory results.

A sandy-loam soil from Nevada was used for growing the plants, both before and after transplanting. Since experiments conducted by growing plants in this soil showed that the disease did not develop on the roots, regardless of whether or not the soil was previously heat-sterilized, non-sterilized soil was used.

The control plants were treated exactly like the inoculated ones, except that plain sterilized barley grain was added to the soil in place of barley on which the fungus was growing.

The plants were grown at a soil temperature of 25–30° C. for 2 months, then removed, and the roots were examined for disease development. In this period, many lesions developed on the roots of inoculated plants, whereas no lesions developed on the roots of control plants (Fig. 1, F). Results typical of those obtained from inoculations are given in table 1.

[4] Identified by John E. Kotila, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

Lesions produced on alfalfa roots, as a result of inoculation tests, are very similar to those that occur on roots under natural conditions at Bard, California (Fig. 1, B, C, D, and E). There can be no doubt, therefore, that *Rhizoctonia solani* is the cause of the disease here concerned.

## DISCUSSION

In the experiments herein reported it has been shown that at a soil temperature of 25–30° C. *Rhizoctonia solani* causes root lesions on alfalfa. It is entirely possible, however, that under this condition there may be other organisms capable of producing similar root lesions, or may at least be effective in furthering the progress of the lesions once they are initiated.

The relation between lesion development and the death of fine roots is

TABLE 1.—*Number and per cent of alfalfa plants with root lesions following inoculation with Rhizoctonia solani*

| Series | Plants inoculated | Results[1] | Per cent infection |
|---|---|---|---|
| Series 1, inoculated | 25 | 23/25 | 92 |
| " 1, control | 0 | 0/13 | 0 |
| " 2, inoculated | 26 | 26/26 | 100 |
| " 2, control | 0 | 0/14 | 0 |

[1] Numerator = number of plants with root lesions.

not at present fully understood. Lesions often develop on large roots where fine roots have grown, but it is difficult to determine definitely whether the lesions are initiated before or after the death of the fine roots. When the disease was first observed, it appeared that some organism was killing the fine roots and then making its way from the fine roots into the larger roots to cause the lesions. However, in inoculation experiments conducted in Nevada soil, it has been observed that in some cases the lesions develop at the base of apparently healthy roots, thus indicating that, occasionally, the lesions are initiated before the roots are killed. Also, it has been observed, with many plants examined during the summer at Bard, that many of the fine roots are dead and those not dead are dark brown; yet no lesions are present on the larger roots. While it is possible that in such cases the disease has not progressed into the larger roots, it appears more likely that *Rhizoctonia solani* is not responsible for all the killing of fine roots on alfalfa in the vicinity of Bard, and that some other factor, or factors, are responsible for the death of many of these fine roots.

It has been known for several years that alfalfa stands are of short duration at Bard, plant mortality being greatest during the latter part of the summer. It seems unlikely, however, that *Rhizoctonia solani* is responsible for all the killing of stands. When plants become so deprived of fine feeder roots and root nodules during the summer, as many of the plants do at Bard, it is possible that they so thoroughly exhaust their root reserves

and become so weakened that they fall an easy prey to weak soil-inhabiting parasites, which are unable to parasitize strong healthy plants. Furthermore, it is known that *Fusarium oxysporum* var. *medicaginis* is present in this area, and it undoubtedly is a factor in the death of many plants.

SUMMARY

A disease of alfalfa roots, herein called rhizoctonia root canker, which occurs on alfalfa roots in parts of southern California and southwestern Arizona, is described and its cause attributed to *Rhizoctonia solani*.

The disease is characterized by dark, slightly sunken areas, which occur on the main tap root and large lateral roots. Lesions usually occur where young roots emerge from the larger roots. They develop mainly during June, July, August, and September, but do not ordinarily develop during the cooler part of the year. Under controlled conditions, the disease has developed abundantly on alfalfa roots grown at a soil temperature of 25–30° C., whereas, it has developed very little, if at all, on roots grown at a soil temperature of 16–18° C.

During the past several years there have occurred serious losses by alfalfa growers on the Yuma Reclamation Project and certain adjoining irrigated areas. The difficulties encountered have been chiefly in the nature of loss of plants requiring frequent reseeding. In extreme cases a high percentage of the plants die within a year from seeding. The disease herein described is believed to contribute importantly to the death of alfalfa plants in the area.

AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY OF NEVADA,
RENO, NEVADA.

# AN ANDEAN DISEASE OF POTATO TUBERS

M. F. Barrus and A. S. Muller

(Accepted for publication April 6, 1943)

While on a trip over the Andes Mountains in Venezuela during November, 1939, the writers, accompanied by G. E. Molinet, then potato specialist of the Agricultural Experiment Station, Ministry of Agriculture and Animal Husbandry of Venezuela, found a peculiar disease of potato tubers, known locally as *buba* disease,[1] in a field and in a storage house at Mucuchíes in the State of Mérida. Specimens had been sent previously from this locality to the Experiment Station at El Valle for examination.

The affected tubers had warty swellings on the surface and were otherwise misshapen, some being cracked (Fig. 1, A). Cuts across them at various places revealed the presence of numerous brownish-black specks throughout the cortex to a depth of 1 cm. or more (Fig. 1, B). Interspersed among these and also occurring throughout the pith were other specks, light-brown or rusty, which appear to be an earlier stage of development than the black ones. No indication of the disease was observed on the roots and stalks, but the plants were mature and most of them were dead at the time. It was not determined whether all tubers of a diseased plant were affected. At least 10 per cent of those in this field showed the disease. Tubers taken to the Experiment Station became reduced within a month to a dry, brown, powdery mass containing numerous sporeballs.

The *buba* disease was found only on the native varieties, Morada and Rosada. The field in which it was found adjoined an area used by the Ministry of Agriculture for testing varieties of cereals. The tubers were being harvested and the badly affected ones were discarded and left in the field. Many of those, affected and not by this disease, bore pustules of powdery scab on the surface, while some of them harbored sclerotia of Rhizoctonia. An examination of tubers in storage disclosed the three diseases.

The disease probably has been present on potatoes of that locality for many years. The altitude of the infested field is approximately 10,000 feet. It was not found in other fields in the State of Mérida, having altitudes of 8000 to 8500 feet, nor in fields in the States of Táchira, Aragua, and Miranda at still lower elevations.

A few references to this disease have been found. Abbott (1, 2) says that it is widely distributed in the mountains of Peru where it is the most important potato disease, sometimes causing complete loss of many of the affected tubers. He ascribes the disease to *Spongospora subterranea,* the

[1] The Spanish word "*buba*" means "pustule" or "small tumor." It was not learned whether the term refers to the disease here described or to powdery scab, which was also present on the same tubers, or to both. The name appears sufficiently descriptive to be applicable to the former. The term "*La roña*" (scab) has been used as the Spanish equivalent of powdery scab.

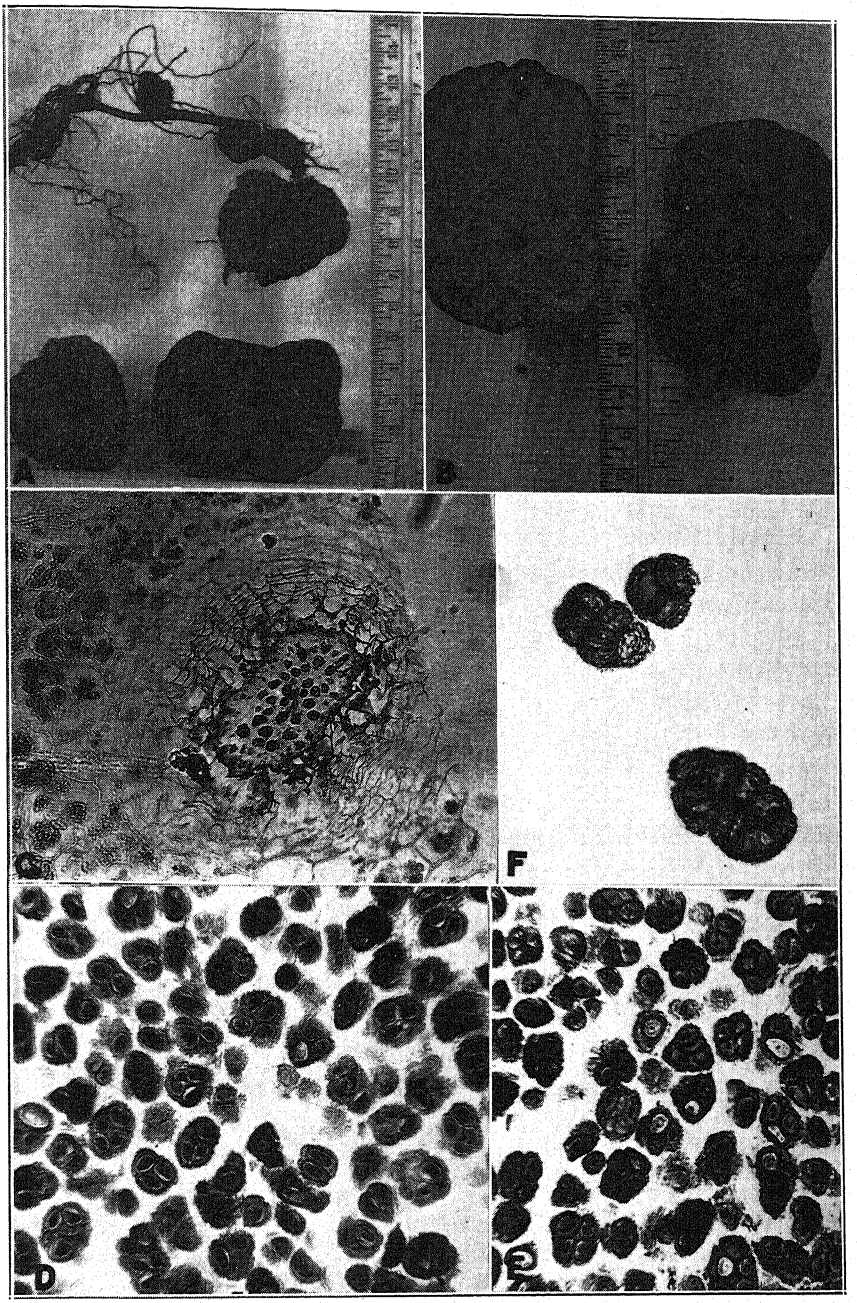



FIG. 1. *Buba* disease of potatoes. A. Malformed tubers; four small ones still attached to stem; $\frac{2}{3}$ natural size. B. A malformed tuber and a transection of it, showing numerous discolored areas in flesh; $\frac{2}{3}$ natural size. C. A section across discolored area in an early stage, showing cork cells separating the lesion from healthy cells; $\times 85$. D. Sporeballs, from a prepared slide of a section of a discolored area. Note their thick roughened wall; $\times 310$. E. Another section of discolored area, showing nucleated cells; $\times 310$. F. Three sporeballs from a mount of spore dust in KOH; $\times 510$.

pathogen of powdery scab. His descriptions and illustrations, however, include both diseases, so that one is uncertain regarding the importance and distribution of the so-called *buba* disease. Chardon and Toro (4) briefly mention a disease of potato tubers characterized by peculiar wart-like protuberances. It occurred on *papa criolla* (*Solanum sp.*), found in a field near Rincón de La Venta, State of Mérida, at an elevation about 10,824 feet. A specimen sent by them to C. L. Shear was determined as *Polysaccopsis hieronymi* (Schroet.) P. Henn., a smut producing galls on stems of *Solanum* sp. in Brazil. A note regarding this specimen occurs in a News Letter of the U. S. Bureau of Plant Quarantine, December 1, 1932 (3). From the description given there and the source of the specimen, one might conclude that the disease on the tubers collected by Chardon and Toro is the same as that collected by us. The sporeballs in our specimens, however, are not those of a *Polysaccopsis*. This has been confirmed by John A. Stevenson and C. L. Shear from specimens we sent them. Stevenson later sent the senior writer a specimen containing sporeballs of the *buba* organism from a tuber of *Solanum tuberosum* (probably *S. andigenum* Juz. et Buk.) collected at Puno, Peru, March, 1940, by J. Soukup, and a specimen of a portion of an affected tuber from Luis Rodriguez Lz. collected near Quito, Ecuador.

Specimens of affected tubers collected at Mucuchíes were taken to the experiment station at El Valle for study. Prepared slides were later made at Cornell University by Ruby R. Rice, then a graduate student, from affected tubers pickled first in 5 per cent formaldehyde solution and later transferred to a preserving solution consisting of alcohol, acetic acid, formaldehyde, and water. The specks, or lesions, consist of subglobose, oval, or irregular-shaped cavities (Fig. 1, C), ranging in size from about 500–1100 $\times$ 300–750 $\mu$, av. 764 $\times$ 522 $\mu$, in the darker lesions to an average of about half this size in the light-brown lesions. A few compound cavities were observed where a narrow strand of tissue separates one compartment from another. Surrounding some of the smaller cavities are 6 to 8 layers of cork cells, but only crushed cells surround the larger ones. Within the cavities are numerous bodies resembling sporeballs (Fig. 1, D), yellowish-brown, mostly subglobose to ovoid, 16–48 $\times$ 12–28 $\mu$, av. 28.9 $\times$ 22.7 $\mu$. These consist of 2 to 8 cells apparently permanently united, 10–16 $\times$ 7–12 $\mu$, av. 12.5 $\times$ 9.1 $\mu$, enclosed by a brown verrucose wall, 2–4 $\mu$ thick. A few sporeballs have only one cell. The cells are also subglobose to oval, somewhat flattened next to contiguous cells, but are variable in shape and size, even in the same ball. Each has a definite wall approximately 1 $\mu$ thick and a well-defined nucleus (Fig. 1, E). The smallest sporeballs contain the fewest cells, and the largest, the most (Fig. 1, F). The material surrounding the cluster of cells forms a kind of wall for the sporeball and extends also between some of the cells. Although it is much thinner there, it appears absent between most of them.

The cavity of the light-brown lesions contains numerous sporeballs that appear to be immature in that they are smaller than those from the darker lesions, a little lighter in color and contain fewer cells. Moreover, these

sporeballs are surrounded in the cavity by a heterogeneous material absent in the darker lesions. The early development of this organism must have occurred when the tubers were younger, since no lesions could be found with an earlier stage than that described. With increase in size of sporeballs, the cavity becomes larger, probably by destruction of the cork cells, which cannot be distinguished in the older lesions. The dry, brown, powdery mass to which affected tubers succumb contains numerous sporeballs released from the cavities.

Soil was infested at El Valle by mixing it with macerated affected tubers and placed in 12-inch pots. Healthy whole and cut Bliss Triumph tubers were planted in this soil. The pots were placed back of the laboratory in partial shade and watered frequently. The plants grew well and, when they had developed tubers 1 to 1.5 inches in diameter, the tubers were examined. A few of the tubers from some of the pots showed the typical lesions of *buba* disease. Uninfested soil produced healthy plants.

The organism, as examined, does not possess all the characters of any described fungus known to us. Before its taxonomic position can be determined with certainty, it will be necessary to germinate the spores. Repeated efforts to do this were mostly unsuccessful, but a mycelium that developed from some of the sporeballs, was cultured. Small portions of the culture were applied to the uninjured and injured surface of young Irish Cobbler tubers. This resulted in a decay of the injured tubers without *buba* disease symptoms.

The writers realize that only a start has been made in a study of the disease and its causal organism. As it is impossible at present for either writer to continue the study, it seems desirable to present at this time the information they have obtained.

CORNELL UNIVERSITY,
ITHACA, NEW YORK.
ESCUELA NATIONAL DE AGRICULTURA,
CHIMALTENANGO, GUATEMALA.

LITERATURE CITED

1. ABBOTT, ERNESTO V. Roña de la papa. *In* "La Roña" y la "Rancha" o "Hielo" de la papa. Estacion Experimental Agricola de la Sociedad National Agraria (Peru) Cir. 7: 11 pp. 1928.
2. ————. La Roña. *In* Enfermedades de las plantas cultivadas en el Peru. Estacion Experimental Agrícola de La Molina (Peru) Cir. 18: 20–21. 1931.
3. Anonymous. Wart smut discovered on Venezuelan potatoes. U. S. Dept. Agr., Bur. Plant Quar. News Letter, Dec. 1, 1932.
4. CHARDON, CARLOS E., and RAFAEL A. TORO. Hunting wild potatoes. *In* Mycological explorations of Venezuela. Monograph of University of Puerto Rico, Ser. B. 2: 38–39. 1934.

# SOME MISCELLANEOUS DISEASES OF MUSHROOMS

A. M. KLIGMAN AND J. S. PENNY

(Accepted for publication April 23, 1943)

## FUSARIUM SPP.

F. C. Wood in England has described a mushroom disease that he attributes to toxins produced by *Fusarium* species (principally *F. solani* (Mart.) var. *martii* (App. and Woll.) Woll. (*F. martii*) and *F. oxysporum* Schl.) in the casing soil (6,7)). The mushrooms fail to mature properly and become stunted, withered mummies with a dry, pithy, discolored interior. Wood has worked largely with the brown form of the cultivated mushroom; he has, however, found the white mushroom, also susceptible, with a resultant rapid rotting.

It was thought at first that a disease similar to this occasionally occurred in beds in the Kennett Square area. Cultures of *Fusarium solani* var. *martii* and *F. oxysporum* were isolated directly from infected pine seedlings.[1] Both species sporulated abundantly on a rye grain medium; hence, inoculated rye grains were used in infection studies. Pure-culture spawn of the brown and white varieties was packed into glass troughs following Wood's technique. The troughs were then cased with infested soil. No diseased mushrooms appeared. On many occasions mushroom beds were infested heavily in the beginning, middle, and end of the crop. Both temperature and humidity were varied. In no case was an infection detected.

In the writers' opinion, Wood's experiments do not show conclusively that *Fusarium* species cause the condition he has described. Wood emphasized the fact that the disease usually occurs under conditions of poor ventilation and a heavy crust in the casing soil. It is possible that these conditions themselves are responsible for the damage. Unfavorable casing soil is known to reduce the yield and increase the amount of "trash" or small mushrooms that do not mature. *Fusarium* species are extremely widespread in soils and may frequently be isolated from normal casing soil. There is yet no positive evidence, at least in America, that their presence in casing soil causes the injury described by Wood.

## MUMMY DISEASE

Mummy disease usually appears during the last stages of the crop and generally is restricted to one or several beds. The diseased mushrooms fail to develop and remain as dry, leathery, mummies. The pileus is small, brownish, frequently tilted to one side with hard abortive gills (Fig. 1, D). The stems are bent, spindly, and shriveled. Internal tissue is tough, leathery, dry, and whitish, and with some discoloration at first in the base of the stem. Longitudinal sections frequently reveal soft discolored pits, streaks, and channels in the tissue of the cap and stem (Fig. 1, B). Later,

[1] Cultures furnished by H. Tint of the University of Penna.

the tissue becomes uniformly discolored and softer (Fig. 1, C). Greenish-gray bacterial slime often lies in the discolored areas. Globules of slime sometime occur between the gills (Fig. 1, E). *Pseudomonas fluorescens* Mig. was consistently isolated from the slime. The base of the stem is frequently swollen and covered with a velvety white overgrowth of mycelium. The disease appears suddenly on a small area and spreads rapidly in both directions until the whole bed is infected.

Shortly after this study was begun, it was learned that Tucker[2] in Missouri had already described the mummy disease without determining the

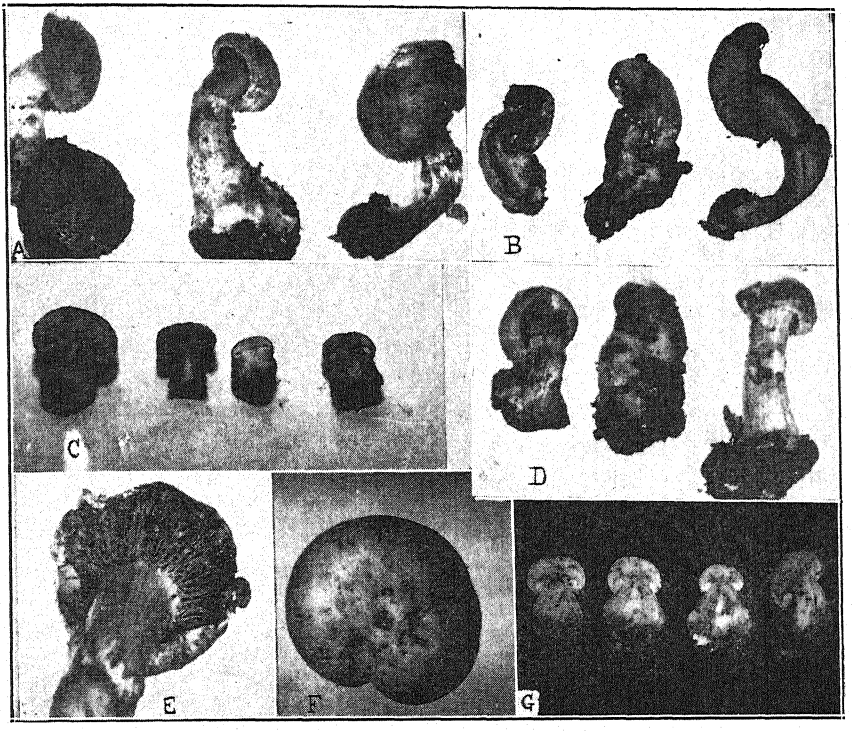



FIG. 1. A. Mummied mushrooms showing swollen base and dwarf cap. B and C. Interior of mummies showing pits and discoloration. D. Characteristic mummies. E. Bacterial slime on gills of a mummied mushroom. F. "Bacterial pit." The injury is probably caused by a mite. G. Interior of mummies collected in Missouri by J. B. Routien.

causal agent (6). He isolated a number of fungi and bacteria from infected mushrooms but was unable to show that any of these caused the disease. The writers have had similar negative results. Organisms isolated from the soil around infected mushrooms likewise produced no evidence of infection in inoculation tests.

Once the disease has appeared, its spread down the bed may be prevented by digging a trench 1 foot wide across the bed. A trench should be

[2] Personal correspondence with J. B. Routien, University of Missouri, established the identity of the Missouri disease with the one in Pennsylvania.

LITERATURE CITED

1. BEACH, W. S. Control of mushroom diseases and weed fungi. Penna. Agr. Exp. Stat. Bull. 351. 1937.
2. BERGEY, D. H., *et al.* Manual of determinative bacteriology. 5th edition. 1939.
3. PAINE, S. G. A brown blotch disease of cultivated mushrooms. Ann. App. Biol. 5: 206–219. 1919.
4. STEINER, GOTTHOLD. *Rhabditis lambdiensis*, a nematode, possibly acting as a disease agent in mushroom beds. Jour. Agr. Res. [U.S.] 46: 427–435. 1933.
5. THOMAS, C. A. Mushroom insects; their biology and control. Penna. Agr. Exp. Stat. Bull. 419. 1942.
6. TUCKER, C. M. A destructive disease of mushrooms. Proc. Mo. Acad. Sci. 3: 71–72. 1937.
7. WOOD, F. C. Studies on "damping-off" of cultivated mushrooms and its association with *Fusarium* species. I. Phytopath. 27: 85–94. 1937.
8. ———. Studies on "damping-off" of cultivated mushrooms and its association with *Fusarium* species. II. Phytopath. 29: 728–739. 1939.

# A WATER SOLUBLE PROTECTANT FUNGICIDE WITH TENACITY[1]

ALBERT E. DIMOND,[2] JOHN W. HEUBERGER,[3] AND JAMES G. HORSFALL

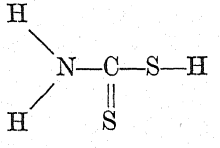
(Accepted for publication August 12, 1943)

Since protective fungicides must resist rain in order to function during infection periods, they usually have been selected on the basis of water insolubility. Water insolubility imposes the limitation that the protectants have to be composed of discrete particles. Discrete particles cannot produce a complete coverage over the surface to be protected. Inevitably, spaces are left between.

A new protectant fungicide has been discovered that is water-soluble. It goes into solution in water and forms a clear yellow liquid, which exhibits a small degree of capillary activity. The combination of the two properties of water solubility and surface activity permits the formation of an invisible film type of coverage. Thus, there are no spaces between particles, since there are no particles, and the capillary activity promotes wetting and spreading actions that assure smaller spaces between droplets than in the case where water is used as a vehicle for carrying particles. When the deposit on the foliage dries, it becomes water-insoluble, hence, resistant to removal by rain.

The compound is disodium ethylene bisdithiocarbamate, the preparation of which has been described recently by Hester.[4] The fungicidal properties of the compound were discovered in January, 1941, when it was selected in the laboratory through bio-assay from 258 organic compounds tested that month. It has been developed under the code number of He175. Chemically, of course, the compound is related to the salts of dithiocarbamic acid, which were originally described as fungicides by Tisdale and Williams[5] and later by Tisdale and Flenner.[6]

Dithiocarbamic acid has the formula:



[1] Approved for publication by the Director of the Connecticut Agricultural Experiment Station. Part of the research project conducted in cooperation with the Crop Protection Institute. Cost of publication of this paper was defrayed by the Crop Production Institute. The writers wish to express their thanks to Dr. W. F. Hester of Röhm & Haas Company, Inc., Philadelphia, Pa., for providing the experimental samples of disodium bisdithiocarbamate.

[2] At present assistant Professor of Botany, University of Nebraska, Lincoln.

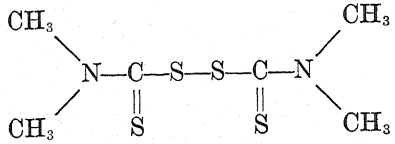
[3] At present Associate Plant Pathologist, University of Delaware, Newark.

[4] Hester, W. F. Fungicidal composition. U. S. Patent 2,317,765. 1943.

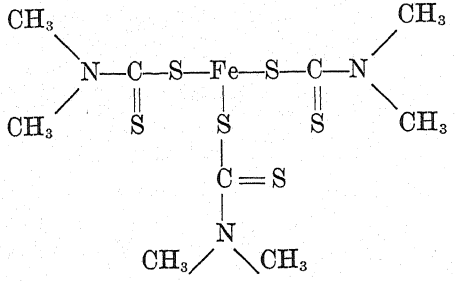
[5] Tisdale, W. H., and I. Williams. Disinfectant. U. S. Patent 1,972,961. 1934.

[6] Tisdale, W. H., and A. L. Flenner. Derivatives of dithiocarbamic acid as pesticides. Ind. and Eng. Chem. 34: 501–502. 1942.

Two molecules can be joined through sulphur atoms by reaction of a dithiocarbamate with sulphur chloride or an oxidizing agent. By way of example, an N-dimethyl dithiocarbamate yields tetramethyl thiuram disulphide:

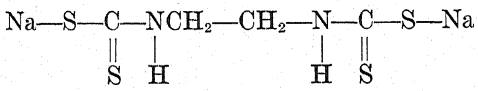


This compound is sold as a fungicide under the names of "Thiosan" and "Arasan." The ferric salt of dimethyl dithiocarbamic acid, ferric dimethyl dithiocarbamate:



is sold to the trade under the name of "Fermate."

Disodium ethylene bisdithiocarbamate:



differs from the above dithiocarbamic acid derivatives in that two molecules of dithiocarbamic acid are joined not through sulphur, but through a divalent hydrocarbon group attached to the nitrogen atom of the respective thiocarbamate radicals.

The important practical difference between the new compound and other derivatives of dithiocarbamic acid is its water solubility, its film-forming ability, and its property of becoming water-insoluble on drying. Both tetramethyl thiuram disulphide and ferric dimethyl dithiocarbamate are particulate protectants. The deposit of the latter is coal black and hence objectionable on many plants. Of course, ordinary sodium dimethyl dithiocarbamate is soluble in water like disodium ethylene bisdithiocarbamate, but sodium dimethyl dithiocarbamate is not capable of becoming insoluble on drying.

A dried deposit of the compound shows the same peculiar double-maximum toxicity curve that was described by Dimond *et al.*[7] for tetramethyl thiuramdisulphide. This peculiar situation permits the compound to have

[7] Dimond, A. E., J. G. Horsfall, J. W. Heuberger, and E. M. Stoddard. Role of the dosage-response curve in the evaluation of fungicides. Conn. Agr. Exp. Stat. Bull. 451. 1941.

three LD50 values, when one is the rule. The material, therefore, cannot be compared to another compound as usual by means of equal LD values.

Tenacity is equally difficult to assay biologically. At present, the common bio-assay for tenacity is to take a ratio of the LD50 values of a compound before and after a standard wash, according to Heuberger's procedure.[8] Since disodium ethylene disdithiocarbamate has three LD50 values, it is possible to have a higher LD50 value after washing than before, and this has happened in testing. Nevertheless, disodium ethylene bisdithiocarbamate resists weathering well in the field.

In our tests disodium ethylene bisdithiocarbamate has given promising control of *Diplocarpon rosae* and *Sphaerotheca pannosa* on roses, *Venturia inaequalis* on apple, *Cercospora apii* on celery, and *Pythium ultimum* on pea seeds. Because of the surface activity of the material, drain-off during spraying occurs sooner than for most protectants. Hence the deposit does not build up as well as that for the particulate protectants. This drain-off is particularly noticeable on easily wetted foliage, like that of potato and tomato. Probably for that reason the material has not given as satisfactory control as expected for *Alternaria solani* on tomato and *Phytophthora infestans* on potato.

The compound has shown little phytotoxicity. There has been a faint suggestion of minute light-colored spots on roses, but their effect was negligible at effective dosages.

The properties of the compound suggest several possible uses. It should prove useful to cherry and peach growers for application to near-ripe fruit, where residues are objectionable. In war time it would be of interest to gardeners who have sprayers without agitators, since the material does not settle out of suspension. It might prove interesting on onions where the foliage is difficult to wet. It should appeal to growers of ornamentals because of the absence of conspicuous residue.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY,
CONNECTICUT AGRICULTURAL EXPERIMENT STATION,
NEW HAVEN, CONN.

[8] Heuberger, J. W. A laboratory biological assay of tenacity of fungicides. Phytopath. 30: 840–847. 1940.

# CONTROL OF SOIL-BORNE ORGANISMS THAT CAUSE ROTS OF GARDEN PEAS

M. F. Babb and G. W. Bohn[1]

(Accepted for publication March 16, 1943)

## INTRODUCTION

In certain types of physiological investigations with garden peas (*Pisum sativum* L.) it is desirable to grow the plants in soil-jar cultures. When somewhat alkaline field soils (pH 7.2 to 7.7) at the Cheyenne (Wyoming) Horticultural Field Station are used for this purpose it is often difficult to secure good stands of plants because of seed and seedling diseases. The diseases also occur in the field at the Cheyenne Station, but seldom reach epidemic proportions. Preliminary pathogenicity work indicates that several diseases may be involved. A virulent oomycete and a pathogenic species of *Fusarium* have been isolated from diseased plants grown in the field and in nontreated field soil placed in jars in the greenhouse. These and certain other isolated bacteria and mold fungi are being studied further. That several organisms may be involved is not unexpected because many species are known to be associated with seedling and root diseases of peas. Harter, Zaumeyer, and Wade[2] listed 9 organisms causing root rots of peas and stated that others may occur. Following repeated failures to obtain good stands from pea seeds treated with the commonly recommended seed fungicides (Ceresan, Semesan, Spergon), two experiments were set up to determine the most suitable method of obtaining good stands of peas in soil-jar cultures by treating the seed or soil, or both, with various sterlizing agents.

## MATERIALS AND METHODS

The tests were conducted in the Station greenhouses during the winter of 1941–1942. Temperatures ranged from 75° F. to 85° F. during the day and from 60° F. to 70° F. at night. The soil used for the treatments came from a station field where the diseases were known to occur. All of the chemical soil treatments (table 1) were applied directly to soil in 2-gal., glazed, earthenware jars provided with drainage holes. Small rocks, covered with a thin layer of sphagnum moss, were placed in the bottom of each jar to facilitate drainage. Three soil samples, designated as Libby Flat, Pole Mountain Creek, and Laramie Airport, came from areas never before cultivated and relatively remote from cultivated areas. These samples were left untreated for comparison with the treated and nontreated station soil. The seed treatments were applied by shaking the seeds with an excess of the specified dust.

[1] Cheyenne Horticultural Field Station, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[2] Harter, L. L., W. J. Zaumeyer, and B. L. Wade. Pea diseases and their control. U. S. Dept. Agr. Farmers' Bull. 1735, 24 p. 1934.

TABLE 1.—*Effect of soil and seed treatments on number of surviving pea plants from 30 seeds sown*

| Treatment rank | Seed treatments | Soil treatments | Planting delayed Series 1 | Planting delayed Series 2 | Average number of surviving plants from 30 seeds sown, 4 replications — Series 1 | Series 2 |
|---|---|---|---|---|---|---|
| | | | *Days* | | *Number* $S_{\bar{x}}$ | *Number* $S_{\bar{x}}$ |
| 1 | None | 40% formaldehyde solution, 1: 50, 1 liter | 14 | 14 | 28.50 ± 0.65 | 28.50 ± 0.65 |
| 2 | Spergon | Sphagnum, 2″ on top of soil | No | No | ..... ..... | 28.00 ± 0.71 |
| 3 | None | Laramie Airport virgin soil, pH 8.0 | No | No | 28.00 ± 0.22 | ..... ..... |
| 4 | None | Live steam, 6 hours | 1 | 1 | 26.50 ± 1.71 | 27.50 ± 1.50 |
| 5 | Spergon | 40% formaldehyde solution, 1: 50, 1 liter | 14 | 14 | ..... ..... | 27.00 ± 1.35 |
| 6 | Spergon | Chloropicrin, 2.2 oz., 4″ deep | 14 | 14 | ..... ..... | 26.75 ± 1.11 |
| 7 | Spergon | Live steam, 6 hours | 1 | 1 | ..... ..... | 26.75 ± 0.75 |
| 8 | None | Sphagnum, 2″ on top of soil | No | No | 25.25 ± 0.75 | 26.50 ± 0.65 |
| 9 | None | Chloropicrin, 2.2 oz., 4″ deep | 14 | 14 | 26.75 ± 0.25 | 24.50 ± 2.10 |
| 10 | None | Libby Flat virgin soil, pH 5.5 | No | No | 25.50 ± 0.50 | ..... ..... |
| 11 | None | Pole Mountain virgin soil, pH 6.5 | No | No | 24.75 ± 0.85 | ..... ..... |
| 12 | Ceresan, New Improved | None | No | No | 26.25 ± 0.75 | 20.50 ± 3.86 |
| 13 | Semesan | None | No | No | 19.50 ± 1.56 | 25.00 ± 1.08 |
| 14 | None | Pyroligneous acid, 5%, 500 ml. | 14 | 11 | 22.75 ± 1.60 | 19.25 ± 1.32 |
| 15 | Spergon | None | No | No | 17.50 ± 2.63 | 21.75 ± 0.85 |
| 16 | None | 40% formaldehyde solution, 1: 6, 105 ml. | 3 | 10 | 11.00 ± 1.08 | 27.75 ± 1.93 |
| 17 | None | Formaldehyde dust,[a] 2 oz., mixed, top 4″ | 3 | 10 | 13.00 ± 2.97 | 23.75 ± 1.38 |
| 18 | Ceresan, New Improved | Live steam, 6 hours | 1 | 1 | ..... ..... | 14.50 ± 0.65 |
| 19 | Cuprocide (Red) | None | No | No | 3.75 ± 2.18 | 20.50 ± 3.23 |
| 20 | None (Check) | None (check) pH 7.2 to 7.7 | No | No | 3.25 ± 0.75 | 18.75 ± 1.80 |
| 21 | Ceresan, New Improved | Sphagnum, 2″ on top of soil | No | No | ..... ..... | 9.75 ± 1.44 |
| 22 | Ceresan, New Improved | 40% formaldehyde solution, 1: 50, 1 liter | 14 | 14 | ..... ..... | 8.75 ± 4.52 |
| 23 | None | Potassium permanganate, 3%, 1 liter | No | 10 | 0.25 ± 0.25 | 6.00 ± 1.58 |
| 24 | Ceresan, New Improved | Chloropicrin, 2.2 oz., 4″ deep | 14 | 14 | ..... ..... | 2.75 ± 1.18 |
| 25 | None | Sucrose, 2 oz., mixed, top 4″ | 14 | 14 | ..... ..... | 2.50 ± 0.65 |
| 26 | None | Acetic acid, 1%, 500 ml. | 14 | 14 | 3.25 ± 1.70 | 1.25 ± 0.48 |
| 27 | None | Sucrose, 1 oz., mixed, top 4″ | 14 | 14 | ..... ..... | 2.25 ± 0.63 |
| 28 | None | Sphagnum ½, soil ½, mixed | No | No | 0.75 ± 0.25 | 2.00 ± 0.58 |
| 29 | None | Sucrose, 4 oz., mixed, top 4″ | 14 | 14 | 1.00 ± 0.71 | ..... ..... |
| 30 | None | Sucrose, 8 oz., mixed, top 4″ | 14 | 14 | 0.00 ..... | ..... ..... |

[a] Formaldehyde dust made from formaldehyde (40%) 1 lb., infusorial earth 6 lbs.

Four jars were used for each treatment, and the treatments were randomized on a greenhouse bench. Thirty peas of the Laxtonian variety were sown in each jar. No record was taken of the time of emergence of the plants. However, emergence was rather uniform in the checks and beneficial treatments, but was delayed in certain of the injurious treatments. The plants were allowed to grow until they were in bloom, when counts were made of those surviving. Two series of tests were made. In the first, either the soil or the seed was treated; in the second series the most successful soil and seed treatments of the first were used alone and in combination. The nature of the treatments together with the results of the two series of tests are summarized in table 1.

## DISCUSSION

The treatments are listed in table 1 in the order of their effectiveness in controlling the diseases as shown by plant survival. The order of listing the treatments was determined in some cases by only one test; but in those instances where a treatment was repeated the order of listing was determined by averaging the results of two tests.

The average plant survival for the checks in the two tests was low or ranked in 20th place among the 30 treatments. There was, however, considerable difference in plant survival between the checks in the two tests. As all seed came from the same package but the soil from different parts of the field, this variation in survival suggests that the diseases were soil-borne and that there was a difference in the kind or abundance of parasites in various parts of the field. There was also a considerable lapse in time between the first and second tests during which the soil was exposed to the action of the sun and drying winds. It is possible that these factors also had some effect on soil infection.

Treatment of the soil in 2-gallon jars with 1 liter of a 1/50 dilution of 40 per cent formaldehyde (1),[3] with live steam for 6 hours (4), or with 2.2 oz. of chloropicrin (9) yielded good stands of peas. Treatment of the seed with New Improved Ceresan (12), Semesan (13), Spergon (15), or Red Cuprocide (19) gave less satisfactory control. Spergon did not affect the efficiencies of the soil treatments (5, 6, and 7). New Improved Ceresan apparently caused injury when used in combination with these soil treatments (18, 22, 24).

The good stands of plants obtained from nontreated seeds in steamed soil (4), in nontreated, virgin soils from Laramie Airport (3), Libby Flat (10), and Pole Mountain (11), and in sphagnum moss (8) in comparison with the poor stands secured in soil from the station experimental field (20) indicate that the diseases were largely soil-borne. There was little indication of any seed-borne diseases. This conclusion is further substantiated by the very poor stands obtained in the soil and sphagnum moss mixture (28), and from the fact that the seed treatments alone (12, 13, 15, 19) failed to control the disease as well as did the effective soil treatments (1, 4, 9).

[3] Figures in parentheses refer to treatment rank given in table 1.

# A NONINFECTIOUS HERITABLE LEAF-SPOT AND SHOT-HOLE DISEASE OF THE BEATY PLUM[1]

C. O. SMITH AND L. C. COCHRAN[2]

(Accepted for publication March 9, 1943)

Several nontransmissible, tissue-perpetuated, virus-like abnormalities have been observed on Prunus plants. Kinman[3] described a trouble on sweet cherry, in California, locally known as "crinkle leaf" and characterized by variegation and malformation of the leaves and a sparse set of small, misshapen fruits. Nursery stock grown from buds from affected trees perpetuated the trouble, and affected orchard trees top-worked with cions from normal trees developed normal tops. Blodgett[4] described a leaf spot of the Italian prune associated with defoliation and severe decrease in vigor. This disorder was similar to that of the cherry in that it was perpetuated in the growth from diseased cions, but was not transmitted to the healthy stocks.

A disease, apparently of the same class as the two listed above, has been under observation for a number of years in seedlings of the Beaty plum. These seedlings were in a planting at the University of California Citrus Experiment Station, Riverside, and were grown from pits from a tree that was purchased from a Texas Nursery Company under the variety name of El Paso. This variety is listed by Wight[5] as a synonym of the Beaty plum, a hybrid between *Prunus angustifolia varians* and *P. munsoniana*. Certain of these seedlings have shown spots and shot holes in their leaves (Fig. 1, A) annually, while others have remained free.

The affected seedlings varied greatly in symptom expression, some being severely and others only mildly affected. Early spring growth on the affected trees appeared to be normal. As the season progressed, the older leaves at the base of the shoots developed translucent flecks that rapidly enlarged. The centers of these spots turned brown (Fig. 1, B), and in the majority of cases, the brown areas separated from the rest of the leaf at a definite line of cleavage and dropped out. The resultant shot-hole effect developed progressively toward the apex of the shoots, and, by midsummer, when growth had become slower, the foliage appeared riddled with holes (Fig. 1, A). The spots and holes ranged from pin-point size to a diameter

[1] Paper No. 492, University of California Citrus Experiment Station, Riverside, California.

[2] University of California Citrus Experiment Station, Riverside, California, and the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[3] Kinman, C. F. A study of some unproductive cherry trees in California. Jour. Agr. Res. [U. S.] 41: 327–335. 1930.

[4] Blodgett, Earle C. A leaf spot of Italian prune perpetuated in budded stock. Phytopath. 30: 347–348. 1940.

[5] Wight, W. F. The varieties of plums derived from native American species. U. S. Dept. Agr. Bull. 172: 1–44. 1915.

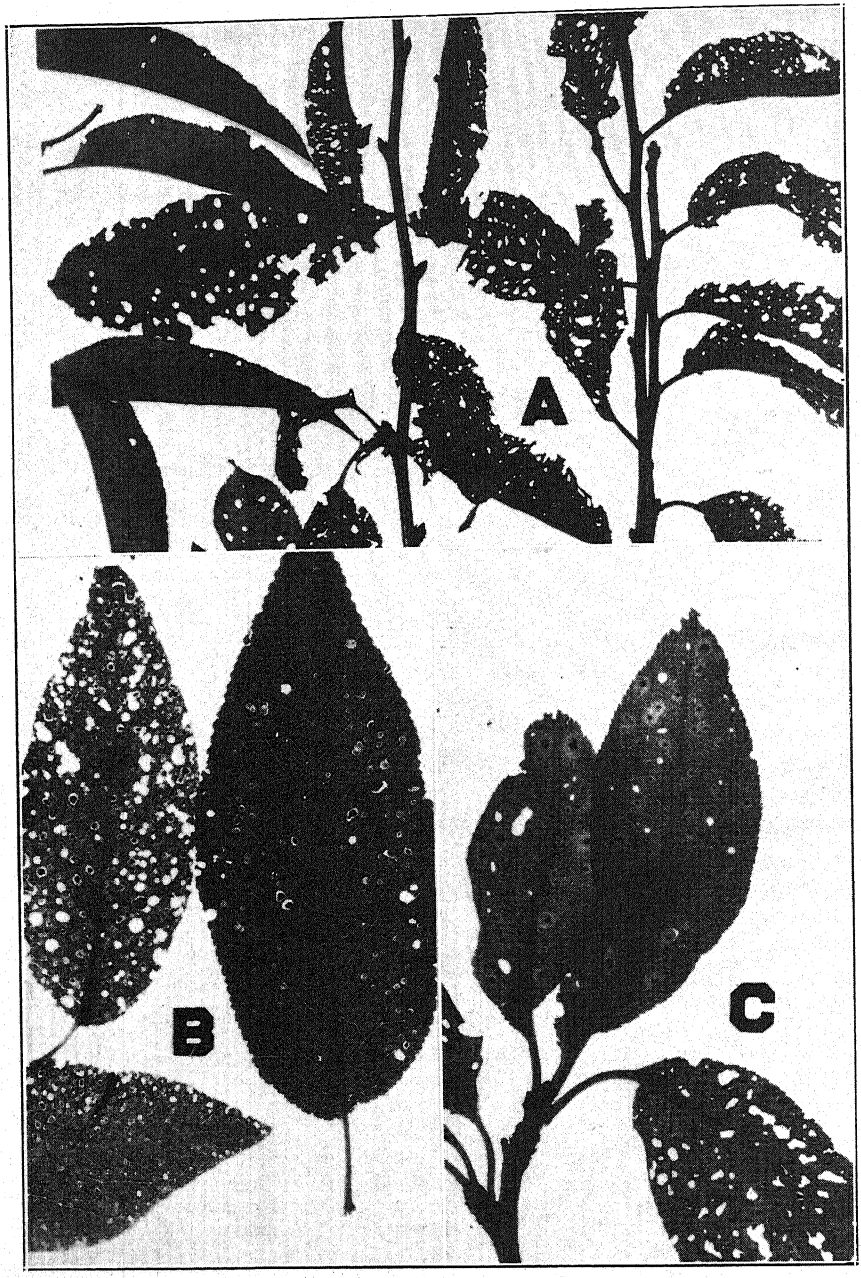



FIG. 1. Leaves of Beaty plum seedling, showing leaf spots and shot holes. A. Shot holes in an advanced stage. B. Shot holes in leaves on shoots from buds that had been taken from a diseased tree and budded into peach stock. C. Shot holes showing the zone about a dark center.

of 1 to 2 mm., the size being inversely proportional to the number on the affected leaf; they were usually circular, but were often of irregular shape where they coalesced or occurred near or in the angles of veins.

The shot-hole condition described above is not macroscopically distinct from that caused by the fungus *Coryneum beijerinckii* Oud., but it develops at a season unfavorable to the fungus. Cultures from the spots yielded no organisms known to cause such injury. This condition is distinguished from a transmissible ring-spot and shot-hole condition described by Cochran and Hutchins[6] on peach, as will be shown from the evidence presented later in this paper.

An attempt was made to inoculate the following species of *Prunus* with buds taken from a severely shot-hole-affected seedling of Beaty plum: *Prunus armeniaca* L., *P. angustifolia* Marsh, *P. communis* Fritsch, *P. cerasifera* Ehrh., *P. hortulana* Bailey, *P. mexicana* Wats., *P. munsoniana* Wight and Hedr., *P. mume* Sieb. and Zucc., *P. orthosepala* Koehne, *P. umbellata* Ell., *P. persica* Sieb. and Zucc., as well as healthy seedlings of Beaty plum. In no case did any of the stocks show symptoms indicating transmission. The initial leaves on shoots from the inoculation buds at first appeared healthy, but they later developed typical spots and shot holes (Fig. 1, C) characteristic of the affected mother tree.

Cions taken from the original parent Beaty plum, which had shown no symptoms, were top-worked onto the seedlings showing the shot-hole condition. None of the growth from the cions showed any shot holes, whereas the leaves of these seedling understocks were riddled with holes.

The nontransmissibility of the disorder, the absence of shot-hole symptoms on the parent tree and on some of the seedlings, and the variation in intensity of symptoms on the affected seedlings suggest that the shot-hole condition is a genetical abnormality that segregates among the progeny. The parent tree, as previously stated, was known to be a hybrid. While the symptom characters of the disease simulate those caused by some viruses, the nontransmissible nature of the trouble precludes its inclusion in the class of known virus diseases.

[6] Cochran, L. C., and Lee M. Hutchins. A severe ring-spot virosis on peach. (Abstract) Phytopath. 31: 860. 1941.

# SEVERE POTATO LATE-BLIGHT INFECTION IN SEBAGO TUBERS[1]

G. H. Rieman and John S. McFarlane

(Accepted for publication April 15, 1943)

The new Sebago potato variety has proved to be moderately resistant to late blight caused by *Phytophthora infestans* (Mont.) de Bary. In repeated tests for 10 years, very few Sebago tubers developed late-blight rot under natural field conditions where susceptible Green Mountain potatoes developed from 20 to 70 per cent decay.[2] In contrast with these findings, exceptionally severe tuber decay caused by *P. infestans* was observed in Sebago potatoes grown in a series of field trials in Central Wisconsin in 1941.

Northern-grown foundation seed stocks of Sebago and Russet Rural varieties were planted side by side in rows through the center of commercial Russet Rural fields on 19 representative sandy soil farms in Central Wisconsin in June, 1941. Most of the fields were neither sprayed nor dusted for blight control, and late-blight infection was first observed on Russet Rural vines in mid-August. On September 1 a general late-blight epidemic was in progress, which continued with increasing severity during this month. The vines of the Sebago variety gradually became infected, but at a much slower rate than did the Russet Rural vines. No frosts had occurred up to the first week in October when the paired rows of Sebago and Russet Rural potatoes were harvested. The vines were still partly green at harvest time at most of the locations. The paired rows of the two varieties were dug by hand during a period of frequent showers. Very little tuber decay was observed in the field. Yield records were taken on five 25-hill replicates in each row and a composite sample of U. S. No. 1 size tubers was set aside for further study. These samples were handled in bushel baskets and stored in a commercial potato warehouse for a period of two months.

Many of the tubers of both varieties became badly decayed and covered with typical mycelium mats and fruiting bodies of *Phytophthora infestans* during the storage period. The stored tubers were washed and carefully examined for late-blight tuber rot. The weights of healthy and of infected tubers were taken for each sample. It can be noted readily from the data presented in table 1 that the Sebago tubers showed more rot than did the Russet Rurals. This is most unusual in the light of numerous convincing reports of high late-blight tuber-rot resistance for the Sebago variety.[3] Furthermore, it was found in cutting the potatoes in the various samples that the extent of decay was much greater in the Sebago tubers than in the Russet Rurals (Fig. 1).

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station as Paper No. 314 from the Department of Genetics.

[2] Bonde, Reiner, F. J. Stevenson, and C. F. Clark. Resistance of certain potato varieties and seedling progenies to late blight in the tubers. Phytopath. 30: 733–748. 1940.

[3] Wisc. Agr. Expt. Stat. Ann. Rpt. Bull. 443: 6. 1939.

TABLE 1.—*Late blight tuber rot occurring in 19 paired trials of Sebago and Russet Rural grown in Central Wisconsin in 1941*

| Grower | Sebago | | | Russet Rural | | |
|---|---|---|---|---|---|---|
| | Healthy tubers | Diseased tubers | Diseased | Healthy tubers | Diseased tubers | Diseased |
| | *lb.* | *lb.* | *Per cent* | *lb.* | *lb.* | *Per cent* |
| 1 | 20 | 30 | 60 | 23 | 28 | 55 |
| 2 | 48 | 1 | 2 | 48 | 0 | 0 |
| 3 | 53 | 1 | 2 | 48 | 4 | 8 |
| 4 | 50 | 2 | 4 | 49 | 3 | 6 |
| 5 | 36 | 10 | 22 | 47 | 4 | 8 |
| 6 | 5 | 46 | 90 | 25 | 28 | 53 |
| 7 | 17 | 35 | 67 | 35 | 13 | 27 |
| 8 | 52 | 3 | 5 | 54 | 1 | 2 |
| 9 | 20 | 31 | 61 | 23 | 28 | 55 |
| 10 | 41 | 9 | 18 | 52 | 2 | 4 |
| 11 | 10 | 42 | 81 | 11 | 39 | 78 |
| 12 | 28 | 25 | 47 | 35 | 17 | 33 |
| 13 | 16 | 34 | 68 | 48 | 4 | 8 |
| 14 | 19 | 28 | 60 | 18 | 32 | 64 |
| 15 | 43 | 10 | 19 | 48 | 5 | 9 |
| 16 | 7 | 44 | 86 | 9 | 43 | 83 |
| 17 | 42 | 7 | 14 | 46 | 5 | 10 |
| 18 | 38 | 14 | 27 | 31 | 20 | 39 |
| 19 | 50 | 0 | 0 | 44 | 5 | 10 |
| Total | 595 | 372 | 38 | 694 | 281 | 29 |

The amount of tuber decay found in the susceptible Russet Rurals was not unusual for the season and agreed with grower experiences in the test area. The question arises as to the cause of the high late-blight tuber-rot infection observed in the Sebago variety in this series of field trials. Varia-

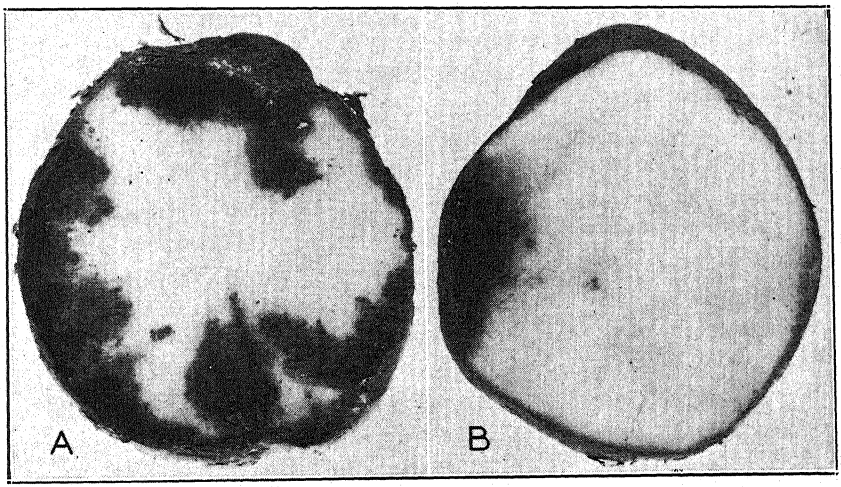



FIG. 1. Typical tuber decay caused by *Phytophthora infestans* in two potato varieties grown side by side in the field and stored for two months under identical conditions. A. Cross section of Sebago tuber showing severe dry rot decay. B. Cross section of Russet Rural tuber showing an average amount of dry rot decay in a standard susceptible variety.

tions in field-plot technique and storage are probably unimportant in dealing with this problem, since the experiment was so conducted as to give each variety identical treatment in the paired trials. The Sebago late-blight susceptibility to tuber rot noted in these tests could have developed in 3 different ways. 1. Tuber infection could have taken place early when the tubers were young and susceptible. However, had this been the case, considerable tuber decay should have been evident at harvest. 2. The Sebago vines were in much better condition than those of the Russet Rurals throughout the epidemic. Under these conditions the Sebago tubers may have been more immature than the Russet Rural tubers and, therefore, more subject to bruising during the harvesting operations. Fresh, actively sporulating growth of the pathogen was present on the vines in most of the fields, and there was ample opportunity for tuber-wound inoculation. 3. A considerable portion of the growing season was exceptionally favorable for the disease. During this extended period more virulent strains of the pathogen may have developed on the partly resistant Sebago host.[4] This would account for both the high percentage of tuber infection and the greater extent of tuber decay found in the Sebago variety.

WISCONSIN AGRICULTURAL EXPERIMENT STATION,
MADISON, WISCONSIN.

[4] Mills, W. R. Adaptive parasitism of Phytophthora infestans. Phytopath. 30: 17. 1940.

Reddick, D., and W. Mills. Building up virulence in Phytophthora infestans. Amer. Potato Jour. 15: 29–34. 1938.

## ANNOUNCEMENT

*Dissolution of the Tropical Plant Research Foundation.*—At a meeting on April 21, 1943, of the Board of Trustees of the Tropical Plant Research Foundation in Washington, D. C., decision was reached to dissolve the Foundation and assign its resources to other organizations more actively engaged in tropical research. The large library, including the life-time collections of the late William Allen Orton, was presented to the Inter-American Institute of Agricultural Sciences at Turrialba, Costa Rica, and dedicated as a memorial to Dr. Orton. Residual funds were given to the National Research Council. It is contemplated that these, together with proceeds from the sale of certain publications given to the Pan American Union, may be used in support of Latin American educational and research programs.

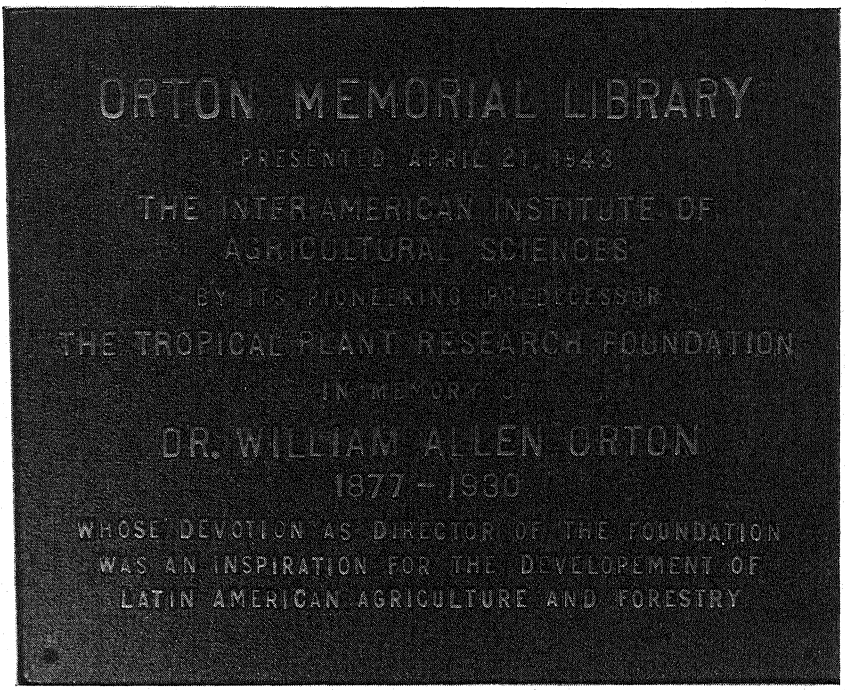



FIG. 1. Bronze tablet in memory of Dr. William Allen Orton presented with his library to the Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica, by the trustees of the Tropical Plant Research Foundation at their final meeting disbanding the Foundation.

The Foundation was dependent upon grants for specific projects from interested business or official agencies, but has been inactive for a number of years. Its extensive researches during the 1920's for the Cuban sugar industry and its agricultural and forestry surveys in several countries have contributed materially toward sound organization of the present inter-

American agricultural research programs. The remaining trustees of the Foundation were L. R. Jones, president of the board, William Crocker, acting director and general manager, R. A. Harper, Herbert Osborn, and S. C. Prescott.

The vision and pioneering activities of Dr. Orton in the advancement of agricultural investigations for improvement of inter-American economic relations were reviewed at the meeting and subsequently expressed on a bronze tablet accompanying his library to Turrialba. As one of the organizers, a charter member and former president of the American Phytopathological Society[1] this tablet presents a fine tribute and enduring reminder of his contributions in a broader field during his later years. Since so few will visit Turrialba in the near future at least, a photograph (Fig. 1) of the memorial tablet is reproduced with this notice.—R. D. RANDS, Rubber Plant Investigations, Plant Industry Station, U. S. Department of Agriculture, Beltsville, Maryland.

[1] Jones, L. R. William Allen Orton, 1877–1930. Phytopath. 21: 1–11. 1931.

# ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE THIRTY-FIFTH ANNUAL MEETING OF THE SOCIETY, COLUMBUS, OHIO, DECEMBER 4 TO 6, 1943

*Blossom Drop of Wax Beans Reduced by Growth Substances.* ALLEN, T. C., ELLSWORTH FISHER, AND A. J. RIKER. Bud, blossom, and pod drop have caused varying yield reductions in wax beans depending on conditions. These physiological diseases in northwestern Wisconsin have been associated with attacks by *Lygus* sp. insects and with long, hot summer days. To prevent abscission layer formation, growth substances were applied. Field dusts with naphthalene acetic acid at 140 p.p.m. increased yields 15 per cent in 1942. This material at 40 p.p.m. gave a 15 per cent field increase in 1943. Other compounds applied as dusts were less effective. In some trials yields were decreased because of chemical or mechanical injury. None of the sprays used was beneficial; some were harmful. Naphthalene acetic acid when too strong induced leaf symptoms suggesting virus infection. To study drop and its prevention by growth substances in greenhouse trials, abscission layers were stimulated in both bean petioles and pedicels by a day's treatment with illuminating gas. Effective treatments started with about one part of gas in 135 parts of air at about 29° C. The growth substances tested were comparable in effectiveness in greenhouse and field, suggesting that perhaps other abscission diseases may be studied advantageously with illuminating gas along with growth substances.

*Action of Clavacin on Certain Phytopathogenic Bacteria.* ANDERSON, H. W. A mycogenous bactericide, named *clavacin* by Waksman, was tested against a number of phytopathogenic bacteria. A strain of *Aspergillus clavatus*, furnished by Waksman, was used in the production of the test clavacin. The sterile, unconcentrated filtrate contained approximately 4 Oxford units per ml. Twenty bacterial species were used, representing 7 species of *Xanthomonas*, 5 of *Pseudomonas*, 5 of *Corynebacterium*, 2 of *Agrobacterium*, and 1 *Bacterium* (*B. stewarti*). A standard strain of *Staphylococcus aureus* was included for comparison. Results were expressed in per cent of filtrate necessary to: (1) kill bacteria (lethal dose), (2) inhibit growth (bacteristatic action) in dextrose broth. On plates the potency was determined by measuring the cleared rings. Lethal dose for various species varied from 2 to 6 per cent. Four species were killed at 2 per cent or less, 9 at 4, and 2 species at 6. Five were not killed at 6 per cent, but bacteristatic action was observed in these at 4 per cent. Bacteristatic action ranged from 1 to 4 per cent, but 10 of the 20 species were inhibited at 1 per cent or less and seven between 2 and 3 per cent. Fifteen of the 20 organisms were *Gram negative*. There seemed to be no relation between lethal or bacteristatic doses and reaction to the Gram stain. *Bacterium stewarti* and *Xanthomonas pruni* were most sensitive. *Staphylococcus aureus* reacted in much the same way as these 2 phytopathogenic, Gram negative bacteria in both cell and dilution methods of testing. Penicillin was entirely ineffective against *Xanthomonas pruni*.

*Pathogenicity for Tobacco of Some Bacterial Isolates Antigenically Similar to Bacterium tabacum.* BEACH, W. S., AND PAUL SACCO. Comparative pathogenicity for tobacco was determined for 62 cultures of bacteria selected from a series of 603, isolated by Reid *et al.* (Penn. Exp. Stat. Bull. 422, 1942) from healthy clover and tobacco and tobacco soils. They reported these 603 isolates among others to have similar agglutination reaction with *Bacterium tabacum* used as the antigen. Following heavy atomization of succulent leaves of Pennsylvania Broadleaf tobacco, 36 isolates, including some from each stated source, produced typical wildfire spots, while 26 isolates gave no apparent infection. A few of the latter, however, showed slight invasion when leaf tissues were water-soaked by a forced spray. These few possibly represent strains of *Bact. angulatum* of low virulence. Isolates showing weak wildfire infection acquired greatly increased virulence by 7 daily passages through either of two liquid media, one composed of mineral salts and 5 per cent asparagin, the other of unheated tobacco juice and 2.5 per cent asparagin. The increased virulence appeared due to greater abundance of the halo-producing toxin. The initially non-infectious isolates acquired no appreciable degree of pathogenicity by passage through these media. This non-infectious character of a large proportion of the isolates is further evidence that similar agglutination may not signify similar pathogenicity. The isolation of *Bact. tabacum* from soil tends to substantiate field observations that this pathogen overwinters in Pennsylvania soil.

*Scab of English Ivy Caused by Sphaceloma.* BITANCOURT, A. A., AND ANNA E. JENKINS. A leaf scab of English ivy (*Hedera helix* L.) has been discovered in southern California, whence specimens from the vicinity of Los Angeles, Mar Vista, and Upland, collected in 1940–1943, have been communicated by H. S. Fawcett. In March of the

current year the disease was found in São Paulo, State of São Paulo, Brazil. The spots are few to numerous, scattered or confluent, occasionally present on veins, usually circular, but sometimes irregular, on the lower leaf surface 1 to 5 mm. in diam., more or less abruptly raised, sometimes depressed at the center, brown, often Verona brown to warm sepia, sometimes ecru drab; above, less prominent and smaller, 1 to 2 mm. in diam., with abruptly raised margin, sometimes purple-bordered to the extent of the spot above. This disease evidently is caused by *Sphaceloma* which is present on the spots and which has been isolated from the specimens from Mar Vista and Sao Paulo.

*Two New Viruses Transmitted by Agallian Leaf Hoppers.* BLACK, L. M. The discovery that of the two agallian leaf hoppers, *Aceratagallia sanguinolenta* and *Agallia constricta*, only the former transmits the New York variety of potato yellow-dwarf virus and only the latter transmits the New Jersey variety suggested that other agallian leaf hoppers might be carrying other yellow-dwarf viruses in nature. To test the hypothesis, *Agalliopsis novella* was selected for investigation. Collections were made with the collaboration of P. W. Oman. When this species was tested on crimson clover, two new viruses were revealed. One of these is transmitted only by *Agalliopsis novella* and the other by this species and also by *Agallia constricta*. The relationships of the new viruses are uncertain.

*On the Survival of Corynebacterium sepedonicum under Various Storage Conditions.* BONDE, REINER, AND S. F. SNIESZKO. Samples of two potato soils from Aroostook County, Maine were sterilized in bottles and inoculated with pure cultures of *Corynebacterium sepedonicum* or crude cultures in form of crushed ring-rot-infected potato tubers. Bottled inoculated soils were buried in November in the ground in Aroostook Farm and left until the end of May. In spite of precautions, sterilized soils in many bottles became infected with various soil microorganisms. From the soils inoculated with pure cultures, *C. sepedonicum* was recovered in 7 out of 18 cases in Washburn loam and in only one out of 18 in Caribou loam. Potato seed pieces infected through placement in some of the soils inoculated with pure cultures, produced ring rot-infected plants. In none of the 36 soil samples inoculated with crushed infected potato tubers could the presence of virulent or viable *C. sepedonicum* be demonstrated. Also, small pieces of cotton and jute potato bags were sterilized and inoculated with pure and crude cultures of *C. sepedonicum* and stored under various conditions since November. Only from samples inoculated with pure cultures were viable and virulent cultures of *C. sepedonicum* recovered 4 to 9 months later.

*The Influence of Guttation Fluid on Pesticides.* CURTIS, LAWRENCE C. Guttation water, collected from different vegetable plants, was shown to contain varying amounts of total solids about half of which was of an organic nature. The guttation fluid, when sprayed on glass slides previously coated with Bordeaux mixture and yellow copper oxide, increased to a marked degree the per cent of inhibition of spore germination of *Macrosporium sarcinaeforme*. Various concentrations of different salts gave similar results. Experimental evidence supports an hypothesis to explain how the copper in Bordeaux mixture is rendered soluble by the guttation water and how this soluble copper enters the leaf to cause injury to susceptible plants or to prevent tipburn on potato plants.

*Deleterious Effects of Guttated Fluids on Foliage.* CURTIS, LAWRENCE C. Both in the field and greenhouse it was observed that 3 things may happen to the guttation drop on plants: (1) it may roll off, (2) it may evaporate, and (3), as most frequently happens on undisturbed plants, it may be sucked back into the leaf. When neutral red crystals were placed in the guttation drop of maize and squash plants, the drop was immediately sucked back into the leaf, and the vascular system of this leaf was stained for a distance of 1 to 2 inches.

An hypothesis is advanced to explain tip burn. When the guttation water evaporates on the tips of the leaves, it leaves various salt deposits that may increase due to a continuous guttation process over a 1-, 2-, or even 3-day period. These deposits may affect the leaf in 2 ways: because of their high salt concentration they may remain and damage the outside of the leaf in the same way that fertilizer does when it is put on leaf blades, or they may go into solution in subsequent guttation water and be sucked back into the leaf where the hypertonic solution kills the cells. New products or changes in the guttation fluid, which are toxic to the internal cells when the fluid is sucked back, are produced either by bacteria, molds, or enzymes.

*Soil Treatments with Sodium Nitrite for Controlling Damping-off and Root Knot.* ELLIS, D. E. Results of greenhouse and plant bed tests in 1942 and 1943 indicate that sodium nitrite is effective in controlling Rhizoctonia damping-off of lettuce. Four and 8 ounces per square yard applied to artificially contaminated plant bed soils 4 weeks before seeding reduced post-emergence damping-off by 71 and 95 per cent, respectively. The

higher rate slightly reduced stands but gave better control than chloropicrin, formaldehyde, or urea. At the same rates, under field conditions, sodium nitrite, applied to soils naturally infested with *Heterodera marioni* 7 weeks prior to planting, reduced root knot infection of bean, okra, squash, and tomato. At the higher rate control was equal or superior to that afforded by chloropicrin or urea. Root knot index readings ranged up to 3.2 for the lower and 1.0 for the higher rate as compared with 56.0 to 71.5 for the controls. Comparable figures on the basis of percentage of plants affected were 16, 3, and 76 to 100, respectively. Counts showed that the treatments reduced stand only in the case of beans. Observations indicate that stand reductions may be largely overcome by earlier application.

*Sodium Nitrite for Root-knot Control.* FELDMAN, A. W., AND LUTHER SHAW. During the past 2 years a number of chemicals have been employed as soil treatments in an effort to find a control for the root-knot nematode (*Heterodera marioni*). Of the chemicals applied, sodium nitrite proved most effective. Under greenhouse conditions, using tomatoes as the host, concentrations as low as 6 oz. per sq. yd. have given 93 per cent control, and 12 oz., 100 per cent control. Plant response to this treatment was quite noticeable in comparison with other treatments and checks. Not only was rapid growth produced, but greener foliage, good fruit set, and well-developed roots resulted. A method of standardizing chemical applications to various soil types was employed. The base exchange capacity and replaceable hydrogen were determined, and sodium nitrite was added in equivalents of 50, 100, 150, and 200 per cent of the replaceable hydrogen. Greenhouse and laboratory tests showed a rapid transition of the $NO_2^-$ to $NO_3^-$, temperature and moisture being important factors. Good growth was obtained when plantings were made as early as 1 week following application of the chemical. Microflora analyses of the soils under field conditions indicate that the chemical is highly toxic to soil organisms.

*Electrophoretic Studies on Sap from Plants Infected with Tobacco-mosaic Virus.* FRAMPTON, VERNON L., AND W. N. TAKAHASHI.

*Expressed Sap of Tomato Plants in Relation to Wilt Resistance.* GOTTLIEB, DAVID. Sap expressed from stems of 3 varieties of tomato retarded the growth of *Fusarium bulbigenum* var. *lycopersici* in proportion to the wilt resistance of the variety. The average yield of mycelial mats in sterile expressed sap were 0.145, 0.110, and 0.065 g., for Bonny Best, Marglobe, and Pan American, respectively. Similar results were observed when the saps were mixed with equal parts of 3 per cent potato dextrose agar and the linear growth measured. After 10 days the average size of the colonies on media from the above-mentioned varieties were 81, 74, and 65 mm., respectively. The retarding material in the sap was stable at 100° C. for 2 hours, and was absorbed by activated charcoal at room temperature. It can be distilled at 95° C. under reduced pressure and the condensed distillate retains its inhibiting properties.

*Seed-borne Diseases of Cereals in Manitoba—A Survey, 1937–1942.* GREANEY, F. J., AND J. E. MACHACEK. During the 6-year period 1937–1942, a large number of samples of wheat, oats, barley, and rye seed were collected each year in Manitoba and examined for the presence of parasites causing seed-borne diseases. Plating tests of the seed showed that, among the numerous organisms isolated, species of *Helminthosporium* (*H. sativum* on wheat, barley, and rye; *H. avenae* on oats; and *H. teres* on barley) were the most important pathogens. Several species of *Fusarium* were commonly isolated, particularly from oat seed, but they were not harmful. Soil-bed tests showed a high positive correlation between the percentage of seeds infected with *H. sativum* and *H. avenae* and the amount of disease in the subsequent seedlings. In wheat, but not in barley, seed infection with *H. sativum* was associated with low germination. Of the wheat samples tested 96.4 per cent were virtually free from surface-borne smut; whereas over 80 per cent of the oat and barley samples carried a smut spore load sufficiently high to make seed treatment necessary. Each year, nearly half (46 per cent) of the Manitoba seed stocks of wheat, and more than 80 per cent of those of oats and barley, required seed treatment for disease control.

*The Effect of Plant-decomposition Products on Root Diseases.* GRIES, GEORGE A. The variable effect of different crop residues on strawberry root rot is known. In the experiments herein reported crop plants were decomposed in 2-gal. crocks. The extracts produced were sterilized by filtration and their toxicity was tested on strawberry and tobacco plants grown in water culture. The sterile extract of red clover, decomposed by its natural microflora, was decidedly toxic to the test plants, whereas the extracts of soy beans similarly produced were innocuous. When these plants were sterilized and decomposed by the microflora peculiar to the other host, the order of toxicity was reversed. This indicated that the evolution of toxic decomposition products is largely a function of the

organisms involved. Variations in the toxicity of extracts from various collections of timothy showed considerable difference in the type of microflora that may be associated with a given crop. The effect of certain crop rotations on the incidence of parasitic diseases was indicated by the toxicity of many of the sterile extracts to pathogenic fungi in culture.

*Juglone (5-hydroxy-1, 4-naphthoquinone)—A Promising Fungicide.* GRIES, GEO. A. Juglone (5-hydroxy-1, 4-naphthoquinone), the oxidized form of hydrojuglone found in members of the genus *Juglans*, is toxic to certain higher plants growing in the proximity of walnut trees. In standardized laboratory tests for fungicidal activity on a series of naphthoquinones and related compounds, juglone most nearly approached the toxicity of the copper in Bordeaux mixture. The L. D. 50 values of freshly extracted or synthesized juglone using *Sclerotinia fructicola* and *Macrosporium sarcinaeforme* as test organisms were 0.19 and 0.27 micrograms per sq. cm., respectively. These data were based on a spore load of 50,000 spores per cu. cm. The L. D. 50 values increased slightly over a 6-mo. period under laboratory conditions, suggesting a tendency for juglone to decompose to by-products of a lower order of toxicity. Greenhouse tests proved juglone to be ineffectual as a contact or stomach insecticide. Although the toxicity of juglone to the root systems of higher plants was confirmed, no foliage injury was evident when it was sprayed on cutinized surfaces. Tests in which juglone was used as a seed protectant indicated that it was extremely deleterious to the non-cutinized surfaces of germinating seeds. In preliminary field experiments, promising results were obtained with juglone for the control of black spot of roses.

*Life History of Hypoxylon pruinatum in Relation to Pathogenicity on Aspen.* GRUENHAGEN, R. H. Aspen, in the Lake States, is used extensively for boxwood, pulp wood, and excelsior, and is a potential source of raw cellulose for explosives and plastics. Stands examined had 10 to 60 per cent of the trees infected with *Hypoxylon pruinatum*, which was found to enter only through bark wounds. The best results from inoculation were secured when the fungus was introduced through a wound near the center of a bruise. An active infection girdled a 4-inch tree in 2 to 3 years and spread vertically 3 to 6 feet. The fungus was seen only in microscopic sections of the cortex, cambium, and outer wood. Conidia were borne on conidiophores at the surface of the canker, as well as on raised mycelial pillar-like structures. Conidia were abundant during spring and early summer. Ascospores were forcibly discharged from April through September out of perithecia in stromata raised above the canker surface. Both conidia and ascospores were wind-borne. Warm, rainy weather, followed by periods of high humidity favored spread of the disease. The organism overwintered both as ascospores and as mycelium. The organism on 2 per cent malt agar grew best at 28° C. and at pH 5.0 to 6.0.

*Effect of Seed Treatment on the Control of Oat Smut.* HANSING, E.D. Quantities of Kanota oat seed inoculated artificially by dusting with chlamydospores and by partial evacuation with a suspension of chlamydospores, and naturally infected seed from the previous season were treated with different fungicides. Each treatment was replicated and the data analyzed by analysis of variance. Seed inoculated by dusting and then treated produced average smutted panicles as follows: checks 38.7%, seed treated with volatile fungicides (New Improved Ceresan, DuBay 1452-C (Ethyl mercury p-toluene sulfonanilide), and formaldehyde) 0.6%, and seed treated with "nonvolatile" fungicides (Arasan, Corona Coppercarb, micronized free-flowing sulphur, micronized wettable sulphur, Spergon, Yellow Cuprocide, and yellow mercuric oxide) 2.7%. All fungicides gave significant control of smut. Seed inoculated by partial evacuation produced average smutted panicles as follows: checks 60.9%, seed treated with volatile fungicides 8.0% and seed treated with nonvolatile fungicides, 63.4%. Thus, only volatile fungicides gave a significant reduction in percentage of smut. Naturally infected seed produced smutted panicles as follows: checks 34.2%, seed treated with volatile fungicides 0.4%, and seed treated with nonvolatile fungicides 5.0%. Although all of the seed treatments reduced the percentage of smut significantly, satisfactory control was obtained only by the use of the volatile fungicides (volatile vs. nonvolatile, odds $> 999:1$).

*Studies on the "Black Point" Disease of Wheat in the United States.* HANSON, E. W., AND J. J. CHRISTENSEN. Prevalence, severity, causes, distribution, symptoms, and effects of the seed discoloration commonly known as "black point" were studied from 1935 to 1942 on 5 to 25 varieties of bread and durum wheats obtained from 13 States. The amount of seed discoloration, the fungus flora concerned, and all other factors studied varied greatly, depending on the year, region, and variety grown. Percentages of discolored seed ranged from 8 to 73 within certain States in the same year; and differences between States were even greater. In general, durums were more susceptible than bread wheats. *Alternaria* spp. were most commonly isolated, although *Helminthosporium* and

*Fusarium* were most prevalent in some regions in some years. In general, prevalence of seed discoloration was correlated positively with prevalence of microorganisms and plumpness of seed, and negatively correlated with percentage of germination and seedling stand. There was no association with test weight or with varietal reaction to leaf and stem rust. The benefits of seed treatment depend on the prevalence of *Helminthosporium* and *Fusarium* rather than on the total microflora. Seed treatments with Semesan Junior increased stands in certain lots from 23 to 67 per cent. (Cooperative investigations between the Division of Cereal Crops and Diseases, U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station.)

*Boron Soil Treatments and the Development of Flax Diseases.* HART, HELEN, J. E. MITCHELL, AND DAVID GOTTLIEB. The addition of boron to soil did not affect development of flax rust and pasmo in numerous field and greenhouse experiments in 1942 and 1943, although promising results in reducing rust had been obtained by other investigators in 1941. The application of 50 lb. or more of sodium tetraborate to sandy loam soil at University Farm, St. Paul, tended to reduce stands, height of plants, and yields of flax. The addition of even smaller amounts tended to increase damage from wilt and Rhizoctonia.

*Effect of Zinc Sulphate-Lime on Protective Value of Organic and Copper Fungicides Against Early Blight of Potato.* HEUBERGER, J. W., AND T. F. MANNS. Preliminary evidence indicated that the addition of zinc sulphate-lime to fungicides might aid in decreasing run-off and in increasing initial deposit and tenacity. In an experiment on Dakota Red potatoes, designed to evaluate the protective value of organic and copper fungicides, zinc sulphate-lime at 1–½–100 was added to 3 of 7 materials. Fungicides used were Spergon (tetrachloro-parabenzoquinone), Fermate (ferric dimethyl dithiocarbamate), U. S. Rubber Co. #604 (2, 3-dichloro-naphthoquinone-1,4), Rohm and Haas Co. He175 (disodium ethylene bisdithiocarbamate), Yellow Cuprocide (copper oxide), Compound A (copper oxychloride) and Bordeaux at a toxicant concentration of 1½–100. Seven applications at 200 gallons/acre were made at approximately 10-day intervals (July 29–Oct. 3) on replicated randomized plots. Records on Oct. 16 showed following control: He175-$ZnSO_4$-CaO, 92%; Yellow Cuprocide-$ZnSo_4$-CaO, 86%; Bordeaux, 85%; Compound A-$ZnSO_4$-CaO, 82%; Yellow Cuprocide, 68%; Compound A, 58%; #604, 54%, He175, 40%; Fermate, 15%; Spergon, 4%; unsprayed, 0%. The possibility of synergism, and of new compound formation in the He175-$ZnSO_4$-CaO combination, is being considered. (Yield records had not been taken when this abstract was submitted.)

*Effect of Organic and Inorganic Seed Treatments on Rate of Emergence, Stand, and Yield of Soy Beans.* HEUBERGER, J. W., AND T. F. MANNS. The following materials were used in a test on the Scioto variety of soy beans to determine if seed treatments were beneficial under Delaware conditions: Spergon (tetrachloropara-benzoquinone); Arasan (tetramethyl thiuram disulphide); Dow #5 (tetrachloroquinone); Dow #6 (2, 4, 5-trichlorophenate); Ceresan (ethyl mercury phosphate). Semesan (hydroxymercurichlorophenol); Yellow Cuprocide (copper oxide). The first 5 were use at 2 oz.–1 bu. and the last 2, Ceresan and Cuprocide, at 1 oz.–1 bu. Treatments were planted in replicated randomized plots on June 25. The percentage stand 5 and 15 days, respectively, after planting was: Dow #5, 46.8% and 86.1%; Arasan, 40.4% and 84.9%; Ceresan, 38.5% and 82.6%; Spergon, 37.8% and 85.2%; Dow #6, 29.8% and 75.0%; Semesan, 28.4% and 64.1%; Check, 28.1% and 65.0%; Yellow Cuprocide, 15.4% and 55.5%. These data show that the materials fell into three classes: (A) accelerated rate of emergence and increased final stand (Dow #5, Arasan, Spergon, Ceresan); (B) Little or no effect on rate of emergence and final stand (Dow #6, Semesan); (C) decreased rate of emergence and final stand (Yellow Cuprocide). Yield data showed that materials in Class A increased yield, that those in Class B had little or no effect, and that those in Class C reduced yield.

*Fire Blight Control by Prevention of Infection.* HILDEBRAND, E. M.

*The Problem of Dry Rot Caused by Macrophomina phaseoli (= Sclerotium bataticola).* HOFFMASTER, D. E., J. H. MCLAUGHLIN, W. WINFIELD RAY, AND K. STARR CHESTER. Macrophomina dry rot ("charcoal rot") is a major pathological and economic problem in corn, sorghums, potatoes, beans, cowpeas, and peanuts in the South. Losses reach 48% in corn and 5–75% in potatoes. New hosts are catalpa, cedar, alfalfa, sudan grass, mung beans and broomcorn. *M. phaseoli* mainly attacks seedlings, causing damping-off, and sub-mature plants, the ensuant root decay resulting in stem-rot, precocious ripening, low yields, and premature death. It is moderately and variably aggressive. Its invasion is favored by devitalization, characteristic of plants subjected to environmental extremes of continental climates, and wounds or attacks of other organisms. It is adapted to high temperatures. The correct name, according to International Rules of nomenclature, and

that now generally accepted by American and British workers, is *Macrophomina phaseoli* (Maubl.) Ashby. Single conidia invariably produce the sclerotial stage. A theory that sclerotia may be immature pycnidia is proposed. Tentatively proposed control methods include cultural practices that increase crop health and vigor, liming of soil for sorghums, increasing the organic matter in soil, and use of resistant crop varieties, which include many sweet sorghums, some kafirs, and certain other sorghums. Resistance is characteristic of some corn hybrids.

*Experimental Hosts of Tobacco-etch Virus.* HOLMES, FRANCIS O. Tobacco-etch virus was transmitted to one or more species in each of 15 families of plants. Among the experimentally susceptible species were several that conceivably might function as overwintering hosts in nature. One of these, a perennial weed, *Physalis subglabrata*, was shown to be naturally infected in New Jersey. Although tobacco-etch virus is serologically and immunologically distinct from tobacco-mosaic virus, as well as much more sensitive to inactivation by heat, a marked correlation was found in regard to responses of plants to the two viruses. The species that proved susceptible to infection by tobacco-etch virus proved susceptible to infection by tobacco-mosaic virus, although the converse was not true.

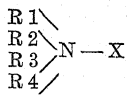
*Spraying Efficiency of New Types of Spray Equipment.* HOPPERSTEAD, S. L., M. W. GOODWIN, AND PAUL L. RICE. The ability of the Speed Sprayer to apply a uniform deposit of spray materials throughout all parts of trees was compared to standard orchard equipment using two 8-nozzle brooms, one operated from the tower and one from the ground, and to a vertical boom attachment using adjustable guns. The vertical boom attachment was in the experimental stage. Twenty-three-year-old Stayman trees were used to which 12½ to 13 gal. of spray mixture were applied per tree per application. Seven spray applications were made. Leaf samples were taken before and after each application, and laboratory analyses made. Data obtained from analyses of samples collected one month after the final spray was applied were as follows: Speed Sprayer—top 213.1, bottom 296.0; Vertical Boom—top 313.7, bottom 297.5; Eight nozzle brooms—tops 269.3, bottoms 278.2, Nonsprayed trees 35.4. Results are expressed as micrograms $As_2O_5$ per sq. in. leaf surface. In addition, samples taken in the tops of the trees within a 5-ft. radius of the trunk gave the following analyses: Speed Sprayer—171.6, Vertical boom—262.3, eight nozzle brooms—185.0. Fruit records collected throughout the season and at harvest indicate close correlation between codling moth control and the leaf analyses. Fruit records were not completely assembled when this abstract had to be submitted.

*The Coverage Factor in Fungicidal Efficiency.* HORSFALL, JAMES G., AND A. D. MCDONNELL. The dynamics of coverage by protectants is not well understood, but a knowledge of them is currently urgent because of the possibility of extending critical fungicides by improving coverage. An effort was made, using the dosage-response technique, to distinguish the probability of hitting a spore (coverage) from the probability of killing it with what hits it. Statistically, a steep dose-response curve means high uniformity. Theoretically, the steeper the slope, the smaller the probability of missing the spore and the more nearly the experiment measures only the probability of killing the organism with what hits it. Field slopes are flatter than laboratory slopes presumably because the probability of missing the spore on a plant is greater than when the spore is placed on a sprayed slide. Laboratory slopes, however, can be steepened and performance of deposits can be improved by increasing spray time. In many but not all field trials on potatoes and tomatoes, slopes were steeper for long than for short spray times. Since run-off increases in the field with spray time, it tends to cancel the practical benefit of the improved coverage by the deposits that are actually retained by the foliage.

*Cationic Phenyl Mercury Compounds as Specific Apple-scab Eradicants on Foliage.* HOWARD, F. L., AND M. B. SORRELL. Two compounds excelling liquid lime sulphur for eradicating *Venturia inaequalis* hyphae and conidia from fruit and leaves without necrosis of healthy apple cells have proved promising in orchard, individual leaf, and laboratory tests. Phenyl mercuric 2′, 2″ nitrilo triethanol lactate (Puratize N5–X) and phenyl mercuric 2′, 2″ nitrilo triethanol acetate (Stearonyx P) are effective at dilutions of 1: 5,000 to 1: 10,000; inexpensive; available in quantity; compatible; and almost equally toxic in acid or alkaline water. In an orchard test of 7 fungicides with spray applications timed according to Rhode Island spray-schedule recommendations, a foliage index on July 20 or 10 randomized replicate trees showed 2% infection on McIntosh and 4% on Baldwin compared with 92% and 34% on respective control trees. A second index on September 2 showed no infection on McIntosh and Baldwin foliage in contrast to 74% and 20% on the respective control trees. Fruit exposed to the Puratize spray were free of Scab. Study of infected attached apple leaves, individually sprayed with the phenyl mercury compounds at dilutions of 1: 5,000 and 1: 10,000, indicates that only the pathogen

and injured suscept cells are killed and not healthy apple tissues. Laboratory toxicity tests against *Macrosporium sarcinaeforme* spores show an L. D. 50 at 3 p.p.m. and a steep slope "dosage response" curve.

*Cationic Quaternary Ammonium Compounds as Fungicides.* HOWARD, F. L., AND H. L. KEIL. The fungicidal properties, phytotoxicity, and detergency of the generic group of chemicals having the type formula



have been studied. R 1, R 2, R 3, and R 4 denote alkyl, alkylene, or aryl groups, at least 1 and not more than 2 of the groups being long-chain members containing 10 to 18 carbon atoms. The remaining 2 or 3 groups are short-chain groups, although one may be a cyclic radical such as a benzyl group. An acid radical, such as a halide, designated by X, provides the cationic property. Examples are dimethylbenzyldodecylammonium chloride, dimethylethyloleylammonium bromide, lactone of carboxymethyldimethyldodecylammonium chloride, dimethylethanoldodecylammonium chloride, dimethyldodecylamine oxide acetate, and dimethyldidodecylammonium chloride. These compounds are water-soluble; colorless; inexpensive; stable, and non-poisonous to higher animals. Laboratory tests have shown the compounds to have an L.D. 50 between 10 and 50 p.p.m. against *Sclerotinia fructicola* conidia and 30 to 80 p.p.m. against *Macrosporium sarcinaeforme* spores with steep slope "dosage response" curves. The effective fungicidal range is from 1: 2,000 to 1: 10,000, while slight flecking will occur on tomato and potato foliage at 1: 1,000 or greater. The compounds have marked wetting and penetrating properties, since they will reduce the contact angle of water from 45% to 70% at concentration of 1,000 p.p.m.

*Gloeosporium venetum and G. necator: Two Distinct Species.* JENKINS, ANNA E., AND C. L. SHEAR. Examination of an authentic specimen of *Gloeosporium venetum* Speg. shows that this fungus, described from Italy in 1879, is not melanconiaceous, but pycnidial in nature; moreover, the host is presumably *Rubus fruticosus* L. and definitely not *R. chamaemorus* L., as originally described. Among sphaeropsidaceous fungi, no species is known definitely to apply to *G. venetum,* although *Phyllosticta fuscozonata* Thuem. also described on leaves has certain characters in common with it. In Ellis and Everhart's description of *G. necator,* producing destructive stem cankers on black raspberry and red raspberry (*Rubus* spp.) in the United States, these authors (1887) state that *G. venetum* "has spores of about the same size but is a foliicolus species." Scribner (1888), recognizing *G. necator* as the cause of Rubus anthracnose, adopted Spegazzini's binomial as an earlier synonym of Ellis and Everhart's new species from America. A recent examination of typical material of *G. necator* shows clearly that this melanconiaceous species belongs to the genus *Sphaceloma* dBy. The separation of *G. necator*[1] from the little known *G. venetum* proves that the earliest records of Rubus anthracnose are actually from the continent of North America and not from Europe as formerly assumed. As late as 1931 Arnaud and Arnaud stated that the disease was uncommon in Europe.

*Adaptation of Protectant-spray Programs to Follow Eradicant Ground Spraying for Apple-scab Control.* KEITT, G. W., AND J. DUAIN MOORE. In non-ground-treated orchards in Wisconsin the lime sulphur-lead arsenate tree-spray programs commonly used for apple scab often fail to control the disease satisfactorily and frequently cause objectionable spray injury. An eradicant ground spray of ½% Elgetol, 600 gal. per acre, applied at about bud-break has been shown to reduce the ascosporic inoculum to a low level and substantially lessen the strain on the protectant spray program. Experiments are now in progress to adapt tree-spray programs to use in conjunction with ground spraying, seeking adequate disease control and reduced host injury. Conditions for scab development were very severe in 1943. Counts on fruit at harvest showed satisfactory control of the disease by various programs sufficiently mild that only minor host injury was occasioned. While conclusions must await further tests, the available data point towards the feasibility of using such programs in Wisconsin on ground-sprayed orchards. In previous experience similar programs have not controlled scab reliably in Wisconsin on non-ground-treated orchards in severe scab years.

*The Spray Program for Cherry-leaf Spot in Relation to Epidemiology, Control, Host Injury, and Fruit Size.* KEITT, G. W., AND J. DUAIN MOORE. Under the severe conditions that commonly occur in Wisconsin, spray programs strong enough to give satisfactory control of cherry leaf spot frequently occasion objectionable host injury and reduce the

[1] The perfect stage of *Gloeosporium necator* is *Elsinoë veneta* (Burk.) Jenkins.

size of fruit. Dilute Bordeaux, or Bordeaux substitutes, tried in 4-spray programs, (1) just after petal-fall, (2) 10 days or 2 weeks later, (3) 2 weeks later, and (4) just after harvest, have not given adequate control in years of severe epidemics. Epidemiological studies and chemical determinations of spray residues indicate that a critical period for control comes between the first and second applications. In 1942 a severe outbreak of the disease occurred in well-managed orchards when a protracted, heavy rain period slightly delayed the second application. Experiments were initiated on programs of graduated dosage in the several applications. In 1943, a season of severe leaf spot occurrence, various programs of this type (*e.g.*, Bordeaux (1) 6–8–100, (2) 3–4–100, (3) 1½–2–100, (4) 6–8–100) gave satisfactory results in disease control. Other data suggest 3–4–100 Bordeaux would have sufficed in application 4. The crop was too sparse for adequate data on fruit size.

*A Foliar Mottle and Necrosis of the Chippewa Potato Associated with Infection by a Strain of the Potato x Virus.* LARSON, R. H. A mosaic of Chippewa characterized by irregular chlorotic-mottle patches in intercostal areas of upper leaves and small scattered necrotic-flecks on older leaves was observed in the field in 1942 and 1943. External and internal root, stem, and petiole tissues of affected plants are normal. Tubers are slightly smaller than normal, but show no pathologic symptoms. Aphid (*Myzus persicae* and *Macrosiphum solanifolii*) transmission tests yielded negative results. Artificially inoculated *Nicotiana tabacum* and *N. rustica* developed small, local ring spots, systemic ring spots and irregular line patterns. Potato seedling 41956 was immune in all tests. Complete protective action of a common mild latent-mottle strain of the potato x-virus was shown on *N. tabacum, Datura metel* and *D. meteloides,* indicating the virus is a variant of the x-virus group. It would appear, therefore, that Chippewa, highly resistant to mild mosaic and a masked carrier of common strains of the x-virus, is subject to infection by an uncommon strain of the latter, which causes a mosaic mottle and necrosis. Infection was secured on Chippewa, Sebago, Triumph, Red Warba and Russet Burbank and on first-generation potato seedlings derived from seed of Katahdin by selfing. Symptoms developed more rapidly and severely at 20° C. than at 24° C., while they were entirely suppressed at 28° C. and above.

*Studies on Ustilago striaeformis from Bluegrass.* LEACH, J. G., AND C. V. LOWTHER. Spores of *U. striaeformis* from mature sori on bluegrass are very difficult to germinate, and artificial inoculations with such spores have produced low percentages of infection. By using spores from young unruptured sori and mycelium in tissues just prior to spore formation, the fungus has been isolated in pure culture on agar. Both sporidial and mycelial growth types occur. Certain growth types produce chlamydospores in great abundance. Spores produced in culture are slightly larger than those produced on plants and less prominently echinulate. A few are abnormal in shape. Spores produced in artificial culture have not yet been germinated successfully. The cytology and infection capabilities of the various cultural types are being studied.

*A Method for Developing an Epiphytotic of Ergot.* LEWIS, RALPH W. In order to induce an epiphytotic of ergot an abundance of spores was so prepared that the spores remained viable in cold storage and on the plant following application. Cultures were grown on sterilized wheat. When the spores formed, the cultures were beaten in a blendor with a minimum of water and screened. An equal weight of beet sugar was added to the thick spore suspension. Tests determined that spores prepared in this way remained viable in cold storage for 3 to 4 months and withstood the drying action of the air for days without a great loss in germinability. The spores germinated on slides when drops of the original or dried suspension were diluted with water. Spore suspensions were applied to rye flowers in bloom with a hand or motor sprayer. 59% of the heads became infected in a plot sprayed 5 times with a knapsack sprayer and 33% became infected in plots sprayed 3 times with a power sprayer. Adjacent control plots were 10% and 3% respectively.

*Fermate Offers Promise in the Control of Frog-eye Leaf Spot (Sphaeropsis malorum) of Apple.* LYLE, J. A., AND LUTHER SHAW. Results of the 1943 season indicate that ferric-dimethyldithio-carbamate (Fermate), used at a 3–5–50 concentration, offers promise as a control of leaf spot (*Sphaeropsis malorum*) of apple. Other fungicides applied included different concentrations of Bordeaux mixture, Tennessee Copper "26," Cupro-K, copper oxychloride, and silver nitrate. Two leaf-spot counts were recorded, the first in the middle of the season, the other during harvest. At each count 500 leaves per tree were examined. Each plot, containing 2 trees per plot, was replicated at least twice. The Fermate plots manifested a total of 10.7 per cent leaf infection for the season with no defoliation. The remainder of the sprayed plots ranged from 41.4 per cent infection for 4–4–50 Bordeaux mixture, to 66.1 per cent for 3–3–50 Tennessee Copper "26" with

some defoliation. The check plots had 100 per cent infection at both counts with half defoliation at the end of the season. It is significant that the Fermate plots exhibited leaves infected with only 1 to 3 lesions per leaf, whereas the other sprayed plots manifested 65 per cent of the leaves with more than 4 lesions. Effective control was not obtained from this material against either bitter rot or blotch of the fruit.

*Natural and Mechanical Injury in Flax in Relation to Seed Treatment.* MOORE, M. B., AND J. J. CHRISTENSEN. A study was made of the relation of seed injury of flax to differential response to seed treatments. Three kinds of injury were of frequent occurrence: mechanical cracking and shattering of the seed coat; discoloration and roughening of the seed coat due to fungi and weathering; and exposure of the embryo due to a natural tendency of seed of certain varieties to split at the germ end. Of the three, embryo exposure was the most common. There was no association between types of injury, but golden-seed varieties were much more subject to embryo exposure than brown-seed ones. Seed lots from Bolley Golden Selection C.I. 976 grown in 31 localities in the United States and Canada differed strikingly in percentage of cracked seed (0–32), of seed with exposed embryos (6–83), and of discolored seed (0–50). The differences in amount of injury between 7 varieties in a single locality were sometimes almost as great as those between seed lots of a single variety grown at different localities. In general, injured seed lots tended to respond to seed treatment more than non-injured; and golden-seed varieties responded more frequently than brown-seed ones. Ceresan and Arasan were about equally effective in increasing the stand.

*Sunscald as a Predisposing Factor to Soft Rot of Early Potatoes in Transit.* NIELSEN, L. W. The early potato crop of North Carolina is harvested during late May, June, and early July. On clear days exposed tubers may be seriously sunscalded. Tubers exposed on the surface of the ground have developed temperatures of 52.2° C., and in bags on open trucks, 50.5° C. Tubers exposed on the ground for 6 hours on July 10, 1942, had bacterial soft rot in excess of 50 per cent after 6 days storage. Potatoes exposed July 21, 1943, for 0, $1\frac{1}{2}$, $2\frac{1}{2}$, $4\frac{1}{2}$, and $5\frac{1}{2}$ hours had the following percentages of rot after storage for 14 days: 3.57, 10.1, 28.0, 74.5, and 78.5, respectively. Using the rapid growth of soft-rot bacteria on cooked potato tissue as an indicator of heat injury, it was found that Irish Cobbler tubers were injured when exposed in a water bath at the following intervals and approximate temperatures: 10 minutes, 51° C.; 30 minutes, 45°–47° C.; and, 60 minutes, 43°–45° C. A tuber (3.0 inches in diameter) exposed to artificial light reached 44.7° C. on the irradiated side after $1\frac{1}{2}$ hours, and was injured to one-third its thickness. In many cases the injury could not be visually detected. These data suggest that some of the soft rot developing in transit is traceable to heat injury prior to packing.

*An Eradicant Treatment for Sooty Blotch of Pears.* PALMITER, D. H. The results of field experiments indicate that a greentip application of Elgetol to Kieffer pear trees will sufficiently reduce the sooty-blotch fungus, *Gloeodes pomigena*, to permit a commercially clean crop of fruit without the aid of summer applications of fungicides. Trees that received a 1% Elgetol application in 1942 produced a crop 80% clean, 13% slightly blotched, and 7% heavily blotched compared with 96% blotched fruit on nonsprayed trees. In 1943 the same treatment, applied to different trees, resulted in 96% clean fruit and 4% slightly blotched fruit. Fruit on nonsprayed trees were 80% infected. Lower dosages considerably reduced the incidence of the disease and may prove sufficient under some conditions.

*Arborvitae Blight.* PLAKIDAS, A. G. A disease of oriental arborvitae (*Thuja orientalis*), referred to locally as "blight" or "fire," has been known in the South for many years. Its chief symptoms are dying and browning ("firing") of leaves, small twigs, or entire branches, and often complete killing of the entire tree. The disease has been attributed to red spider, drought, excessive summer heat, and winter injury. The varieties Berkmann's Golden and Baker are most severely affected, but the disease occurs also on many others. Similar symptoms also occur on the Italian Cypress (*Cupressus sempervirens*). A fungus, apparently a *Cercospora*, has been found constantly associated with the disease. Inoculations with pure cultures have produced it in typical and severe form. Infection also has been obtained by placing conidia-bearing twigs on healthy plants. The fungus has been reisolated from the inoculated plants and resembles somewhat *Cercospora sequoiae* and *C. sequoiae* var. *juniperi*, but differs from these in length of conidiophores and rate of growth in culture. It probably is a distinct species. Perithecia of *Mycosphaerella* sp. are often found on diseased twigs, either alone or in association with *Cercospora*. Ascospore isolates appear similar to *Cercospora* isolates in culture, but inoculations with these have not produced infection thus far.

*Use of a Ferric Dimethyldithio Carbamate and Talc Dust to Combat the Phomopsis Blight of Eggplant.* PORTER, RICHARD P. A single season's field results indicated that

a 10–90 Fermate (Ferric dimethyl dithio carbamate) talc-dust treatment was slightly more effective than a 20–80 copper-lime dust in controlling the leaf-spot symptoms of Phomopsis blight. During the early onslaught of the disease it gave significantly better protection in preventing fruit rot. The plants treated with the Fermate dust also outyielded those dusted with copper-lime.

*The Incidence of Apple Scab and Cedar Rust on Wealthy Apples and Their Effect on Fruit Development in Minnesota.* SHARVELLE, E. G. Apple scab and cedar rust are the most important apple diseases in Minnesota. During the summer of 1943 a maximum fruit infection by scab of 92 per cent and a maximum fruit infection by cedar rust of 60 per cent were recorded on Wealthy apples; however, the introduction of a new spray schedule and spray timing service resulted in controlling all of the major diseases and insect pests. Detailed studies were made on the effect of apple scab and cedar rust on the size and weight of apples. Severe scab caused an average reduction of 20 per cent in size and of 43 per cent in weight. Very light infection by scab caused a reduction in size of approximately 8 per cent. Cedar-apple rust was found to cause an average reduction in size of approximately 8 per cent and a reduction in weight of infected fruit of 24 per cent.

*Cultural Variation in Single Ascospore Isolates of Sclerotinia fructicola (Wint.) Rehm from Cherry Plum Hybrids.* SHARVELLE, E. G., AND SHAN-MING CHEN. Attempts to isolate complete sets of ascospores from single asci of *S. fructicola* were unsuccessful until the conditions influencing ascospore discharge were determined. Maintaining the ascus material at 15° C. for 4–10 hours, then transferring to room temperature for two hours, appeared to stimulate ascospore discharge. Sixteen lines obtained from 2 asci fell into 4 distinct groups on the basis of cultural characteristics, sensitivity to sulphur fungicides, and pathogenicity on apple fruits.

*Use of Potato Juices for Cultivation of Corynebacterium sepedonicum.* SNIESZKO, S. F., AND REINER BONDE. Potato filtrates were prepared by expressing juice of mature tubers or stems and leaves. Some juices were prepared after preliminary freezing of tissues; others were treated with sulphites to prevent darkening of the juice. The juices were first centrifuged and then sterilized by filtration through the Seitz No. 1 sterilizing filter sheets. For the cultivation of *C. sepedonicum* the filtrates were used alone, or with addition of media, phosphate buffers, or agar. The filtrates obtained from the potato varieties Katahdin, Green Mountain, Sebago, and President supported abundant growth of *C. sepedonicum.* Less satisfactory as media were varieties believed to be resistant to ring rot and designated as U. S. seedling varieties, .055, 47102, and 886. Two U. S. seedling varieties, *i.e.,* 46952 and 870, supported little or no growth of *C. sepedonicum.* Results indicated some coincidence between resistance of the potatoes to ring rot and abundance of growth of *C. sepedonicum* on filtered potato juices; the variety President seems to be an exception. There apparently is little if any correlation between the pH of the juices and the growth of *C. sepedonicum.*

*Races of Puccinia graminis avenae in the United States.* STAKMAN, E. C., M. N. LEVINE, AND W. Q. LOEGERING. Although races 1, 2, 5, 7, 8, 10, and 12 of *Puccinia graminis avenae* have been found in the United States, only the first three have heretofore been present in important amounts. Consequently, the oat varieties Richland, susceptible only to 8 and 10, and White Tartar, susceptible only to 7 and 12, have been generally resistant in this country and were, therefore, used as resistant parents in a breeding program. In 1940 and 1941, however, there were indications of increasing prevalence of races 8 and 10; and in 1943 race 8, widespread geographically, comprised about 12 per cent of all racial isolates, and caused appreciable rust on Vicland, Boone, Tama, and other hitherto resistant varieties, mostly derived from Victoria × Richland crosses. This race and the other dangerous races, 7, 10, and 12, were found first in barberry-infested areas. Race 8 may or may not become abundant in the near future, depending on conditions affecting its persistence and spread. Certain generally virulent races, notably 4 and 6, have not been found in the United States, although reported occasionally from Canada. (Cooperative investigations between the U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station.)

*Comparison of Soil Fumigants for the Control of Root-knot Nematode.* STARK, F. L., JR., A. G. NEWHALL, AND BERT LEAR. The efficacies of several liquid nematocides were compared in a commercial greenhouse by injecting the fumigants 4 in. deep, 10 in. apart in replicate plots. In fall treatments of heavily infested soil ethylene dichloride, a mixture of methyl bromide-ethylene dichloride-carbon tetrachloride (1–6.5–2.5) (Dowfume Br–10), and chloropicrin (Larvacide) all gave highly significant control of nematodes and increased yields on a spring crop of tomatoes as compared to checks and a furyl nitro-

ethylene dust treatment. The methyl bromide mixture at 5 cc. per injection gave better control of nematodes than chloropicrin at 2 cc. and ethylene dichloride at 15 cc., but yields were about the same for each treatment.

In a set of spring treatments on soil less heavily infested chloropicrin-methyl bromide mixture (1–3), chloropicrin, methyl bromide-ethylene dichloride-carbon tetrachloride mixture, and chloropicrin-ethylene dichloride mixture (1–9) in descending order all significantly reduced nematode injury. However, there was no significant difference in the yields.

When 3, 5, and 7 cc. dosages of the methyl bromide-ethylene dichloride-carbon tetrachloride mixture were compared, it was found that increasing the dosage markedly reduced nematode injury, but the effect on yield was scarcely significant.

It is concluded that where nematodes are severe all treatments will give an economic net return. At the rates employed treatments can be listed in ascending order of their cost as follows: methyl bromide-ethylene dichloride-carbon tetrachloride mixture, ethylene dichloride, chloropicrin-ethylene dichloride mixture, chloropicrin, and methyl bromide-chloropicrin mixture.

*The Coverage Effect of Sulphur on the Control of Apple Scab.* STODDARD, ERNEST M., AND WENDELL D. HENRY. The effect of coverage on control of apple foliage scab was studied by applying four dosages of sulphur per tree in 2 gallonages. The per cent of infection, plotted as probits, showed a linear relationship to the logarithm of the dose of fungicide. Doses applied in high gallonage gave a steeper slope than those applied in low gallonage. Statistically, a steep slope suggests low variability, or in this case better coverage of each unit of leaf area. This should improve the probability of hitting any given spore. With different slopes, the curves crossed, so that the largest dose per tree gave better control when applied as low concentration-high gallonage, and the smallest dose per tree gave better control when applied as high concentration-low gallonage. At the highest dose, therefore, the probability of hitting a spore with the high gallonage-low concentration seems to be of greater consequence than the probability of killing the spore with high concentration-low gallonage. At the lowest dose, on the other hand, the probability of killing the spore with the high concentration-low gallonage is greater than the probability of hitting it with high gallonage-low concentration.

*Fermate for Control of Early Blight on Tomato.* TAYLOR, CARLTON F., W. H. CHILDS, AND J. G. LEACH. As a tomato spray in 1942, Fermate (ferric dimethyl dithiocarbamate) at 2 lb.–100 gal. proved superior to 80% tetramethyl thiuramdisulphide (2–100), wetable Spergon (2–100), Cuprocide (1¼–100), and Bordeaux mixture (8–8–100) in preventing foliage injury caused by *Alternaria solani.* Yield data were limited to the weight of green fruit remaining when the foliage was killed by frost, fermate having twice the yield of any other treatment at this period.

Fermate, diluted in Pyrax AAB, was tested as a dust on tomatoes in 1943. Data based on individual-plant estimates rated the percentage of green foliage in early October in descending order as follows: 10% Fermate, 5% Fermate + 10% Sulphur, 5% Fermate, 2.5% Fermate, and 6% copper oxychloride sulphate. Yield differences in ripe fruits were non-significant throughout the season, but the quality of the fruit was noticeably better on the plots with less foliage injury.

The Fermate–Pyrax AAB mixture is mechanically well-adapted to dust application.

*The Influence of Known Chemicals on the Initiation of Pathological Growth and Symptoms Resembling Those from Certain Viruses.* THOMAS, JOHN E., AND A. J. RIKER. The effect of 31 known chemicals has been studied on sunflower, marigold, tomato, Paris daisy, velvet leaf, *Kalanchoe,* and *Bryophyllum.* The chemicals were commonly mixed in a lanolin paste and applied to the decapitated stem tips of plants 1½ to 2 inches above inoculations with the attenuated (A6–6) strain of the crown-gall organism, *Phytomonas tumefaciens.* The results from over 2300 chemical treatments and over 4800 inoculations indicated that 12 of these compounds could more or less regularly "activate" tissue about the attenuated bacteria. The most active compounds were indole-butyric acid, alpha-naphthylacetamide, and beta-naphthoxyacetic acid. Other active compounds were p-chlorophenoxyacetic acid, alpha-naphthalene-acetic acid, indole-3-aetic acid, indole-propionic acid, alpha-naphthalenepropionic acid, tryptophane, 2-chloro-5-nitrobenzoic acid, 2-bromo-3-nitrobenzoic acid and phenoxyacetic acid. In general, the other common growth-substance responses noted included axillary bud and abscission layer inhibition, aerial root stimulation, stem thickening, epinasty, gall formation and leaf distortions. Some leaf malformations appeared very similar to certain ones induced by viruses, but they were not transmissible and plants eventually recovered. Variations in growth substance responses were found with different hosts and with different chemicals.

*The Bacteriophage Reaction as a Means of Quick Identification of Pathogenic Bacteria.* THOMAS, R. C. The need for a quick identification method to confirm the presence

of a bacterial plant pathogen frequently has been felt. A study made of 10 plant and 11 human pathogens, as well as of several saprophytic forms of bacteria, indicates that the phage reaction can be used for identification purposes. A phage was prepared for each species of bacteria and tested against all of the other species. Phages appear to show as much specificity of reaction as do diagnostic sera. A few interesting relationships among species were revealed, which suggest further study.

*Tobacco Leaf-spot Bacteria on Roots of Pasture Plants.* VALLEAU, W. D., E. M. JOHNSON AND STEPHEN DIACHUN. Earlier studies have shown that *Bacterium tabacum* and *B. angulatum* multiply on the roots of several crop plants and weeds and may overwinter in this way on the roots of cover crops planted after tobacco. More recent studies demonstrate that *B. angulatum* may persist on the roots of cover-crop plants (wheat and crimson clover) for at least 21 months after tobacco harvest. Inoculations with soil cores containing roots from bluegrass fields in which tobacco beds were prepared proved that the organisms were present, presumably on the roots of some pasture plants, before the diseases appeared in the plant bed. *B. tabacum* also was obtained from weed roots in a field, prepared for setting tobacco, at setting time.

*Charcoal Rot of Irish Potatoes.* WATSON, R. D. Irish potatoes in Eastern Texas often rot in the ground or in storage. This condition was particularly severe in 1943, with losses of 15 to 20 per cent of the crop; some growers lost more than half their crop.

During the past season, the primary causal agent of this tuber rot proved to be *Sclerotium bataticola.* The fungus often enters the tuber at the stolon end. Eyes and enlarged lenticels are also points for infection. The diseased stolon is ashy-grey due to embedded sclerotia. In the tuber the fungus produces a semi-wilted, somewhat watery black rot, which is generally shallow. If no secondary infections occur, the tuber develops into a leathery mummy. This seldom occurs, since one or two secondary rots usually help complete the tuber breakdown. A soft rot, caused by *Erwinia carotovora,* and a dry rot (*Fusarium* sp.) are secondary rots associated in the final stages with charcoal rot.

The losses are closely correlated with high temperatures and high soil moisture—the chief epiphytological factors.

*Effectiveness of Monocalcium Arsenite as an Eradicant Spray Against Sclerotinia laxa.* WILSON, E. E.

*The Chlorophenates as Eradicant Sprays Against Sclerotinia laxa and Coryneum beijerinckii.* WILSON, E. E. The major weakness of sodium tetrachloro and pentachlorophenates as eradicant sprays against *S. laxa* and *C. beijerinckii* appears to be their failure to destroy the mycelium of the fungi inside the host tissue. Although the materials may destroy most of the conidia the mycelium produces new conidia within 2 or 3 weeks. Applications of the sprays as near as feasible to the critical periods for infections (blossoming and leaf-production stages of the host) lessens the chances for the fungi to produce a timely supply of inoculum, but increases injury to the host. Addition of an emulsible oil and a wetting agent increased the penetration of the chlorophenates into host tissue, and to some extent improved their eradicant effect. In field tests on almond trees, sprays composed of 6 pounds of these materials to 100 gallons of water plus 0.5 per cent oil and 8 oz. to 100 gallons of a wetting agent, destroyed from 77 to 97 per cent of the sporodochia of *S. laxa* and prevented immediate production of a new crop of conidia. The effect on the disease was not observed because of blossom injury. The same sprays reduced leaf infection by *C. beijerinckii* 99 per cent. Copper salts of trichlorophenate and tetrachlorophenate were less effective against both fungi than the sodium salts.

*Control of Tomato Anthracnose.* WILSON, J. D. All copper-containing fungicides are comparatively ineffective in anthracnose control. Fermate may give a high degree of control but must be properly used as to timeliness, concentration, rate of application per acre, etc. It is very important that the first application be made early enough, *i.e.,* about July 20 to 30 for cannery tomatoes in northern Ohio, or when fruits of the first cluster are approximately half-grown.

Protection with Fermate continues as long as 40 days after the last application, in early schedules. Since fruits ripening in late September were pollinated approximately 50 days earlier, it seems possible that protection may result from absorption of something from the Fermate by the host plant. Other possibilities are that the fungicide reduces the inoculum present in the surface soil and in fruit lesions, or, less likely, that the fungus is kept from foliage and stems of the tomato plant.

Detached fruits and fruits on defoliated plants are highly susceptible to infection. Sprays that injure the host plant promote fruit infection. Dusts seem as effective as sprays in checking fruit loss. There is some variation in varietal susceptibility, but all commercial varieties used experimentally have shown heavy infection under certain field conditions.

*The Present Status of the Fixed Coppers as Fungicides.* YOUNG, H. C. During the past several years a study has been made of many fixed copper compounds to determine their role as fungicides. Results of laboratory and field tests indicate that considerable variation exists in both effectiveness and injury. The general groups, used in these comparisons, were: (1) the basic copper chlorides, (2) the basic copper sulphates, (3) the copper oxides, and (4) a miscellaneous group.

It was found that the basic chloride and oxide groups were, in general, somewhat more injurious to foliage than the basic sulphate or several of the miscellaneous group, but were also somewhat more effective as fungicides. It also was found that most fixed copper compounds adhered to foliage poorly, which factor may be responsible for the moderate effectiveness of the sulphate group. Many diluent and adhesive materials have been tried but no generally effective one was found.

There is a tremendous variation in the particle size (1 to 75 microns) in the various commercial fixed-copper products. This factor alone influenced adhesiveness and also handicapped the standardization of dusts and their application. It was found that the micronization of a commercial tribasic copper sulphate greatly improved it fungicidally, as well as its physical condition for application.

*Factors Influencing the Production of Local Lesions by the Potato Yellow-dwarf Virus on Leaves of Nicotiana rustica.* YOUNKIN, S. G. It has been shown that variations in environmental conditions, size of inoculated leaves, and inoculation procedures may influence the number, disposition, size, and appearance of the local lesions induced by the potato yellow-dwarf virus on mechanically inoculated leaves of *Nicotiana rustica.* Optimum temperature for local lesion production was 75 to 80 degrees F. At lower temperatures the time required for lesion development was excessive. At higher temperatures the size and character of the lesions were altered. Maximum numbers of local lesions resulted when plants were placed in complete darkness for 12 hours immediately after inoculation. Leaves of *N. rustica* produced by rapidly growing plants and measuring 9 cm. × 13 cm. yielded more local lesions than either (1) leaves measuring 8 cm. × 12 cm. and less or (2) those measuring 13 cm. × 17 cm. and more. Uniform distribution of lesions over the inoculated leaf surface was obtained only with leaves of optimum size. Application of 600-mesh carborundum to the leaves, before inoculation, increased the yield of local lesions. Washing of leaves immediately after inoculation failed to affect the number of local lesions when inoculum consisted of crude sap from infected plants that had been diluted with water.

*Mechanism of Action of 8-hydroxyquinoline.* ZENTMYER, GEORGE A. 8-hydroxyquinoline has long been known to have bacteriostatic and fungistatic properties, and is extensively used as an antiseptic. The mechanism of action of this organic is, therefore, of interest. The fact that 8-hydroxyquinoline is a useful agent in quantitative analysis for precipitating many minor elements (Cu, Mn, Fe, Zn), suggested the theory that it acts fungistatically by precipitating one or several of these elements so that the microorganisms cannot use them. *In-vitro* experiments show complete inhibition of growth of several fungi (*Ceratostomella ulmi, Fusarium oxysporium, F. lycopersici, Penicillium* sp.) by 8-hydroxyquinoline at a concentration of 1–10,000, at pH 6. If the acidity is increased to pH 3, by 0.1 N HCl, growth of these fungi is unaffected by the presence of 8-hydroxyquinoline. When a culture growing at low pH (3.0), in the presence of 8-hydroxyquinoline, is filtered through a Berkefeld filter and the filtrate is readjusted to pH 6 and reinoculated with the fungus, inhibition of growth occurs. These findings are in agreement with the fact that the inner complex metal-hydroxyquinoline salts are known to be soluble in solutions of mineral acids. This cyclic organic chemical thus may be acting as an antivitamin, in the case of minor elements which are connected with vitamin formation and action.

*Logarithmic-Probit Relation of Spore Dosage and Response in Dutch Elm Disease.* ZENTMYER, GEORGE A., JAMES G. HORSFALL, AND PHILIP P. WALLACE. In general, biological responses follow the logarithm of the dosage. This principle has been applied in studies (1) on distance of local spread of the Dutch elm disease, and (2) on spore dosage in relation to disease incidence and to amount of wood discolored in inoculated elms. In (1), the percentage of trees diseased, plotted as probits, decreased with the logarithm of the distance from a central source of inoculum. In (2), dosages of 10, $10^2$, $10^3$, $10^4$, $10^5$, and $10^6$ spores per tree were injected into elms; the disease response as percentage wilt per tree, plottd as probits for this "all-or-none" type of reaction, increased directly with the logarithm of the number of spores injected. In the graded type of response, the total length of wood showing vascular discoloration followed a linear relation to the logarithm of the number of spores introduced into the tree. The spore-dosage data also provide a sound explanation for the failure of the expected percentage of elms in heavily infected areas to show disease symptoms. The disease apparently will not build up sufficiently to develop symptoms when the tree receives only a small dose of spores.

# STUDIES ON CYTOLOGY OF USTILAGO CRAMERI[1]

C. S. WANG

(Accepted for publication May 22, 1943)

## INTRODUCTION

*Ustilago crameri* (smut of millet) is one of the few smuts known to complete its life cycle on artificial substrata (28); therefore, it affords an exceptional opportunity to study the nuclear behavior in saprophytic as well as in parasitic stages.

It has been shown frequently that new physiologic races of certain smut fungi may arise through hybridization or mutation (8, 19, 23). Nevertheless, many of the investigations have not dealt with the actual mechanism concerned in the behavior of the nuclei, that is, the cytological basis for the possible origin of new races. It is well known that meiosis in most smuts occurs during chlamydospore germination, but it is not known at what stages during germination the reduction of chromosomes takes place (10, 12, 23). Moreover the nuclear behavior of the hyphal cells in either parasitic or saprophytic stages varies in different species, and in the same species at different stages of development (15, 24, 25). Therefore, the writer investigated these phases of the problem as thoroughly as possible.

Practically, the resistance or susceptibility of the host to smut fungi is determined by the presence or absence of smutted kernels, but actually this is not proof that the fungus can or cannot penetrate into the host. In fact Brefeld (5, 6) and others (16, 20) thought that the hyphae of certain smuts grew so slowly that they were unable to reach the meristem before the surrounding stelar tissue hardened. Recently, from a histological study, Western (30) concluded that there are three different bases for resistance in oats smut: a reaction of the epidermal cell wall which prevents penetration, necrosis of the host cells, and a retarding effect on the growth of mycelium within the host. Since there is virtually no information on the relationship of *Ustilago crameri* to the host plant the writer also investigated this problem.

The literature on the cytology and host parasite relations in the smuts has recently been adequately reviewed (12, 13, 15, 22, 29, 30) and therefore will not be repeated here, but literature which is pertinent to particular phases of this study will be referred to whenever necessary.

## EXPERIMENTAL RESULTS

### Cytology of Ustilago crameri

*Nature and Type of Chlamydospore Germination.* The chlamydospores of *Ustilago crameri* Kcke. are ovoid to subspherical and 8 to 11 μ long.

[1] A portion of a thesis presented to the Graduate School, University of Minnesota, July, 1937, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Published as Paper 2086 of the Journal Series of the Minnesota Agricultural Experiment Station.

Under ordinary conditions the spores are capable of germination without dormancy and have been shown to remain viable for at least 62 years (27). On germination, a promycelium emerges through a gap in the spore wall. It may branch considerably, but no sporidia have been observed. Sometimes 2 or more promycelia may arise from a single chlamydospore.

*Meiosis.* In order to study the nuclear behavior during germination, spores were dusted with a camel-hair brush, on a thin film of 1 per cent malt agar or 1.5 per cent potato-dextrose agar, spread upon slides, then put in a sterilized moist chamber until the desired stage of germination had been reached. The material was then killed in Flemming's weak solution, washed, and stained in iron-alum haematoxylin.

The mature chlamydospore of *Ustilago crameri* contains a single nucleus (Pl. 1, Fig. 1), which is diploid. This also is generally true for other smut fungi (9, 17, 18, 24, 29). In *U. crameri* at germination, the first division of the diploid nucleus takes place either in the chlamydospore or in the promycelium (Pl. 1, Figs. 1 to 10). Unfortunately, the early stages of the prophase were not observed. Later, 4 small deeply staining units, which are probably the chromosomes, can be seen in the nuclear membrane, but the chromosomes are too small to allow one to see the actual split. Subsequently, the chromatin masses move apart and contract into 2 daughter nuclei (Pl. 1, Figs. 1 to 4). In the meantime a papilla appears with dense cytoplasm (Pl. 1, Fig. 4). The papilla, which has grown gradually toward the wall, then breaks through it and finally passes outward, forming the promycelium. At the same time the diploid nucleus migrates into the young promycelium and divides (Pl. 1, Fig. 7). The nuclear division in the promycelium is more clearly visible than that in the spore, but it still has not been possible to observe the early prophase of the division. The stages from late prophase to the end of the telophase are shown in plate 1, figures 7 to 11. A typical telophase figure with a single strand of fibers between the two daughter nuclei and the newly forming septum is shown in plate 1, figure 10.

The second division quickly follows the first; in fact, a resting stage is rarely found. In plate 1, figure 12, is shown a 2-celled promycelium with 2 nuclei, each nucleus containing 2 chromosomes (haploid number for *Ustilago crameri*) with a more or less indistinct nuclear membrane. The subsequent stages are shown in plate 1, figures 13 to 15. It is evident that in the prophase of the second division (Pl. 1, Fig. 12) only 2 chromosomes are shown, and at the end of this division there are 2 chromosomes in each daughter nucleus (Pl. 1, Fig. 14). It appears, therefore, that reduction in number of chromosomes has taken place at the first division. In contrast to this, the reduction occurs at the second division in the promycelia shown in plate 1, figures 16 to 18, as there are 4 chromosomes at the beginning of the second division (Pl. 1, Fig. 16). Variations in the chromosome conditions are shown in plate 1, figures 19 to 22, and 23 to 24. In figures 19 to 22, there apparently is no reduction in chromosome numbers either at first division or second division. Particular attention is called to figure 21, because

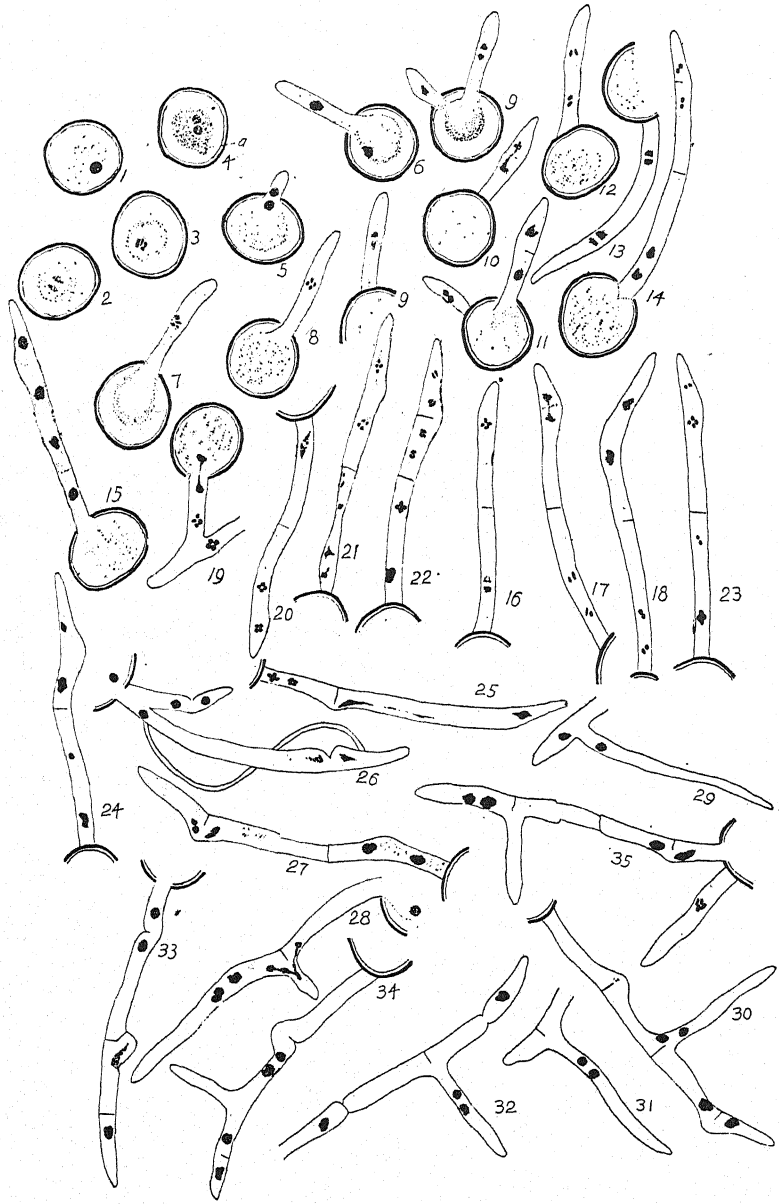



PLATE 1. Fig. 1. A mature chlamydospore with a diploid nucleus. Figs. 2–4. Different stages of the first division in the chlamydospores. In figure 4, *a* is a papilla. Figs. 5–6. Showing the migration of a nucleus from the chlamydospore to the promycelium. Figs. 7–11. Various stages of first division in the promycelium, showing the division figure. Figs. 12–14. Various stages of second division, showing reduction in chromosome number in first division. Fig. 15. A normal promycelium with four haploid nuclei, after two divisions. Figs. 16–18. Reductions in chromosome number in second division. Figs. 19–24. Abnormal reduction division. Figs. 25–32. Initiation of dikaryophase by "knee joint" between two adjacent cells, showing migration of nucleus. Figs. 33–34. Nuclear migration and association, slightly different from figures 25–32. Drawn free-hand; approximately ×1,000.

the nucleus is now in the third division, with 4 chromosomes still distinctly visible, and the spindle fibers are clearer than any others so far observed. In plate 1, figure 23, it will be seen that there are 2 nuclei with 4 chromosomes each and 2 with 2 each; consequently, there are 2 large nuclei alternating with 2 small nuclei in the same promycelium (See also pl. 1, Fig. 24).

The frequency of fusion between the 2 middle cells and the 2 end cells of the promycelium support cytological evidence that reduction in chromosome number may occur in either the first or second division. Therefore, in order to obtain more definite information on the frequency of segregation in either division, observations were made on promycelial cell fusions. Chlamydospores were germinated in potato-dextrose agar at 25° C.; after 72 hours, 300 counts were made on the frequency of cell fusion. The actual ratio of the middle to adjacent end cell fusion was 38.7 : 61.3 as compared with the theoretical ratio 37.5 to 62.5 (3 : 5) if the frequency of segregation in the first division and in the second division were equal.[2]

Bauch (2) and Hüttig (14) have shown that temperature has considerable influence on the time of chromosome reduction in certain smuts. Four different temperatures were tested for their effect on the time of chromosome segregation. The results are given in table 1 from which it can be seen

TABLE 1.—*Effect of temperature on segregation of factors for sex in Ustilago crameri, as indicated by the fusion of promycelial cells*

| Temperature, ° C. | Fusion | | | |
|---|---|---|---|---|
| | Middle cells | | Distal cells | |
| | Actual number | Per cent | Actual number | Per cent |
| 15 | 115 | 37.8 | 189 | 62.2 |
| 20 | 118 | 41.0 | 170 | 59.0 |
| 25 | 130 | 38.7 | 206 | 61.3 |
| 30 | 121 | 38.9 | 190 | 61.1 |

that the ratio of middle cell fusions to adjacent end cell fusions is approximately 3 : 5 at all temperatures. This also indicates that the ratio of segregation of sex factors in the first division is about equal to that in the second division and that the ratio was not modified materially by temperature.

## Origin of Haploid Colonies

It is difficult to isolate haplonts in *Ustilago crameri* because no sporidia are produced. The writer attempted to obtain haploid lines by the method described by Christensen (7) for *Ustilago tritici* (Pers.) Rostr. To isolate haploid lines of *Ustilago crameri*, single chlamydospore cultures were made on hanging drops. As soon as the cultures could be seen with the naked eye,

[2] If segregation for sex factors occurs at random in either the first or second division, then the frequency of segregation of sex factors in the first and second division should be equal, unless crossing over occurs. However, 25 per cent of the cell fusions of the second division should resemble those that occur when segregation takes place in the first division, that is, the fusion of the two middle cells and two end cells of the promycelium, hence the 3 : 5 ratio.

the resulting small colonies were immediately transferred to agar tubes and finally to 2 per cent malt agar in 250-cc. flasks to which enough water had been added to cover the surface in a thin film. After 2 to 3 days the flasks were shaken vigorously to bring about fragmentation of the hyphae, in the expectation that single haploid cells might become isolated in the film of water on the agar. When colonies developed, further isolations were made in the usual manner. By this method a number of haploid colonies were obtained.

It is known that a dicaryotic hypha of smut may dissociate and give rise to haploids (4, 10, 12). It seems possible that sectors in culture, which are not uncommon in *Ustilago crameri,* may in some instances originate by means of nuclear dissociation. In one case 4 different sectors were obtained from a single chlamydospore culture and these, in subsequent tests, proved to be haploid (Fig. 1). Altogether, 47 haploid lines from 8 chlamydospores were isolated from sectors.

In testing the compatibility or sexual reaction of these haploid lines on a susceptible host, no infection resulted when inoculations were made with single lines. Some of the paired combinations, however, resulted in 5 to 10 per cent infection. Different paired combinations of these lines also were observed microscopically and various stages of fusion were observed between some of them (Pl. 2, Fig. 42 to 45).

*Initiation of Dicaryophase.* In *Ustilago crameri,* after meiosis, each promycelial cell is usually haploid in nature but no sporidia are produced. Initiation of the dicaryophase usually results from fusion between 2 adjacent or nonadjacent cells of the same promycelium, but in certain cases fusion between promycelial cells of different chlamydospores also occurs (Pl. 1, Fig. 25 to 35 and Pl. 2, Fig. 36 to 42). The migration of the nucleus from one cell to another, that is, the initiation of dicaryophase, always takes place as soon as the special fusion structure is formed. The detailed process of migration of the nucleus can be traced quite clearly in plate 1, figures 27 to 35. After the dicaryophase is once initiated, it appears to be relatively persistent (Pl. 2, Fig. 38). However, in certain cases the newly formed dicaryophyte may revert to the haploid condition and develop quite well. Apparently, *U. crameri* is primarily homothallic but potentially heterothallic.

The dicaryophase in most smut fungi is of comparatively long duration. It generally starts shortly after meiosis and continues until caryogamy during chlamydospore formation. In *Ustilago crameri,* whether growing parasitically or saprophytically, the hyphal cells are mostly binucleate. Usually they branch and develop very rapidly after entering the host. The younger hyphal cells are so full of cytoplasm that it is hard to determine where the septa are.

As the mycelium grows, uninucleate and multinucleate cells appear more abundantly. Aberrant nuclear behavior is quite common in many different

species of the smut fungi, and even at different stages of development of a species, although the reason is not entirely clear (3, 8, 18, 22, 24).

The significance of clamp connections in the Hymenomycetes has been studied quite thoroughly, while in the smut fungi very little is known about

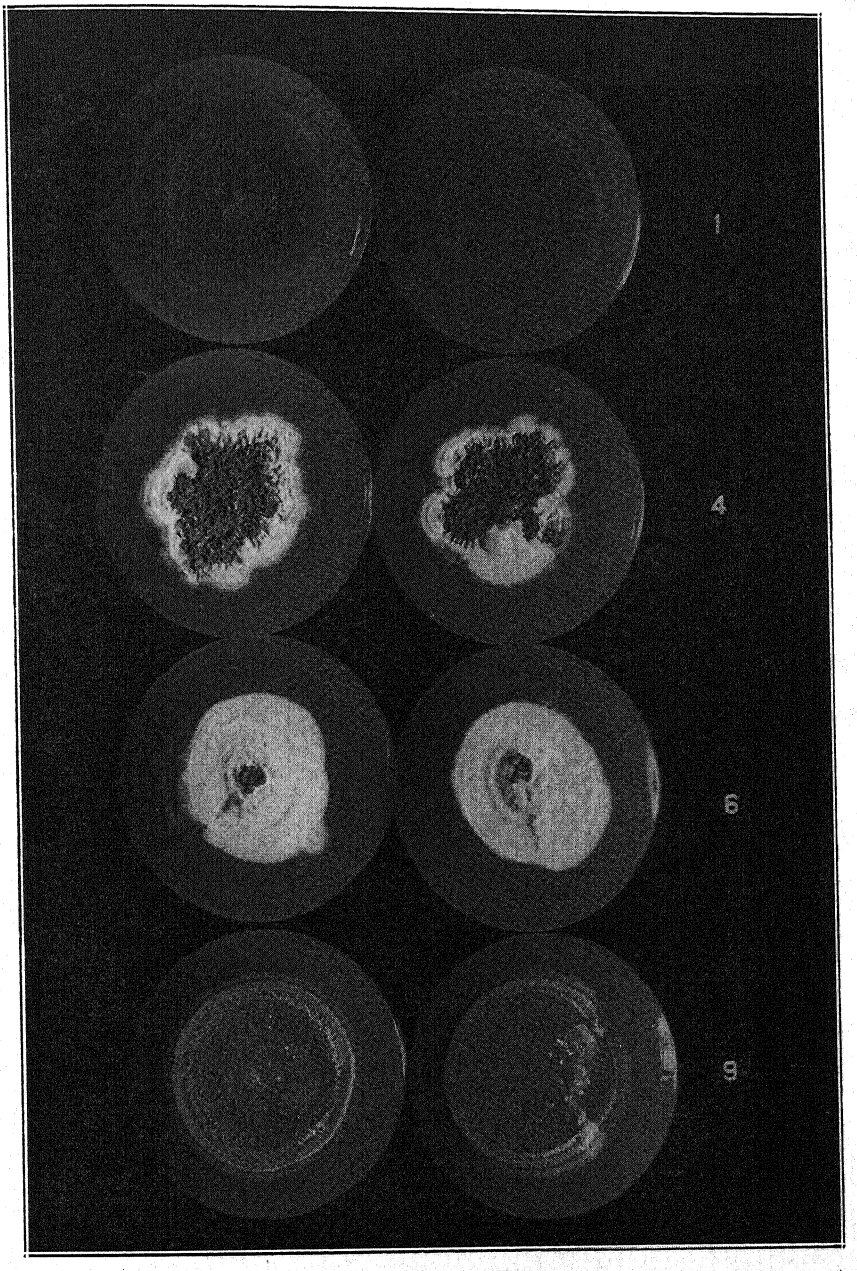



FIG. 1. Duplicate colonies of four haploid lines of chlamydospore XIII showing cultural characters that have persisted since the lines were isolated.

them. Some smuts form typical clamp connections; others form none, or only atypical ones (8, 12, 21, 22). In *Ustilago crameri,* clamp-like connections are often present on the mycelium in the host. Observations indicate that they probably are abortive hyphae.

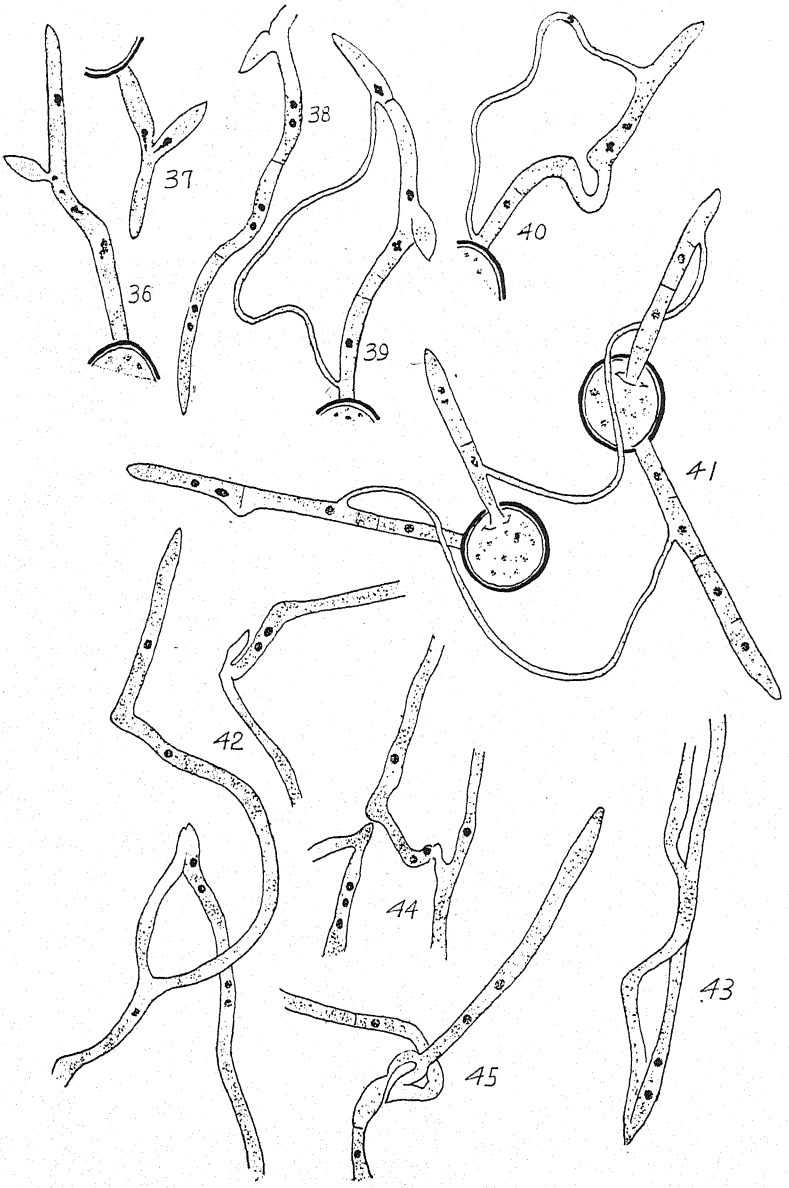



PLATE 2. Fig. 36. A sporidiumlike body. Fig. 37. Initiation of dicaryophase between two adjacent cells, showing nuclear migration. Fig. 38. A hyphal tip branching out from a promycelium, showing the binucleate condition. Figs. 39–40. Initiation of dicaryophase in non-adjacent cells, showing a nucleus in fusion tubes in fig. 40. Fig. 41. Initiation in dicaryophase between two promycelial cells of different chlamydospores. Figs. 42–45. Various stages of hyphal fusions and nuclear migrations between compatible lines. Drawn free-hand; approximately × 1,000.

*Nuclear Fusion and Chlamydospore Formation.* In most smut fungi the true diplophase does not begin until chlamydospore formation, and caryogamy occurs on maturation of the chlamydospores (9, 17, 18, 24). Under favorable conditions chlamydospores of *Ustilago crameri* may be formed in any part of the host plant, except the roots, within 15 days after seed germination. However, they usually are formed only in the ovary of the young spikelet of the ear. At the beginning of spore formation the hyphae, composed of binucleate cells, are both intra- and intercellular. When they reach the cells of the ovary they branch rapidly, just as in the early stages of infection. The mycelial branches, which are either uni- or binucleate, then become twisted, interwoven, and grouped into cluster-like aggregations. This is quite similar to what has been reported by various investigators for a number of other smuts (8, 9, 17, 29). The walls of the mycelial cells soon begin to gelatinize, and the cells in their sorus primordium continue to grow and swell until the process of gelatinization is complete. In the young flowers these sorus primordia may be rather numerous, sometimes coalescing and destroying the entire flower. Meanwhile, the hyphal branches appear to break up into irregular segments lying in the matrix, but the number of nuclei in such cells is not distinguishable. A little later, the gelatinized membrane disappears, and the nuclear behavior in these segments is clearer. At this time the cells change in shape and size, probably during or just before the time of nuclear fusion. Eventually, the cells enlarge, round up, form walls, and become mature chlamydospores. Apparently the spores sometimes may be formed in an intercalary manner also.

*Nature of Infection.* Although Kühn (see 6) and Brefeld (6) observed and recorded the time and place of infection in *Ustilago crameri,* there is no definite information on the nature of infection. This problem was investigated by the writer, particularly in relation to the nature of resistance.

In order to study the early stage of infection in millet seedlings, seeds of a susceptible variety, Kaifeng No. 142, and a resistant one, Nanking No. 31, were treated with formaldehyde in the usual manner and inoculated by dusting with a collection of chlamydospores from China. The inoculated seeds were placed between water-saturated pieces of cotton and incubated in a moist chamber at 25° C.

During germination, materials were studied in both temporary and permanent mountings. For examination of living materials, stripped epidermis or parts of the young seedlings were taken on 7 successive days, stained with safranin and gentian violet, and mounted in water. Other materials were killed, fixed, and stained at the end of the 2nd, 3rd, 5th, and 7th days after inoculation.

Materials for sectioning were fixed in either Flemming's weak solution, Nawaschin's solution, or Bouin's solution in the modifications suggested by Allen (1). To hasten penetration, the preparations were placed in a partial vacuum until the materials sank. After fixing, the materials were imbedded in paraffin and cut into sections 4 to 10 μ thick. Flemming's triple stain,

Heidenhain's iron-alum haematoxylin, Thionin and orange G., or safranin and fast green were used to stain young infected seedlings.

Twenty-four hours after the seeds had been put under favorable conditions for germination the young radicle and the plumule had emerged. Forty-eight hours after germination, most of the promycelial cells of the germinating chlamydospores on the seeds had started to fuse and infection appeared to take place during or shortly after that time.

In an effort to locate the place of infection, many inoculated seedlings, both of resistant and susceptible varieties, were examined 48 hours after germination. No difference in initial penetration into the two varieties was observed, the fungus entering the resistant variety (Nanking No. 31) as well as the susceptible one (Kaifeng No. 142). The fungus can enter any part of the seedlings, but seems to prefer the coleoptile. However, the germ tubes sometimes can penetrate the root hairs, and may grow through the root completely.

As the hypha reaches a cell wall in the coleoptile, after initial infection, it forms a spherical tip closely appressed to the cell wall. In certain cases, the part of the cell wall in contact with the hypha is stained a deep red with the triple stain, as is true also in oat smut (16). There were some indications that the hypha enters through a hole in a softened wall, but penetration by means of a fine thread or a thicker hypha also could be seen. These observations, together with the results of other investigators, led to the general conclusion that some unknown chemical changes may take place in the host-cell walls when the hyphae penetrate them.

## Distribution of Mycelium in Host Tissue at Various Stages of Development

*Susceptible Variety—Kaifeng No. 142.* After the infection tube enters the host, it develops and branches rapidly within 24 hours. Occasionally, the hyphae in the epidermal cells are larger than those in the later stages, but this is not known to be universally true. In order to locate the mycelium at each stage of development, a series of longitudinal sections were made. In the coleoptile of a 2–4-day-old seedling the mycelium had not extended beyond the third layer of the coleoptile. Mycelium proved much more abundant in the central and upper parts of the coleoptile than in the lower part. This is possibly due more to the elongation of the coleoptile than to the selective penetration of the fungus. In 4-day-old seedlings, the new hyphae appear to be narrower than the original ones. In the meantime, the mycelium grows up and down, crosses the space between the coleoptile and the first leaf or passes directly to the meristematic regions and the vascular system and eventually enters the growing point. From now on the mycelium is either intracellular or intercellular in the vicinity of the growing point. It has been found in the adventitious roots in 21-day-old plants.

In the studies of the resistant variety Nanking No. 31, it was found that there is no difference whatever with respect to the initial penetration. Twenty-four hours after the germination of the seedlings, no branch devel-

opment of the infecting hyphae could be observed. They have been found in the second or third layer of the coleoptile in 5-day-old seedlings. Later, they disintegrate into short, isolated, intracellular segments. Of all the 19 seedlings examined, only 2 were found in which hyphae had penetrated to the meristematic region of the growing point. Further development was not followed. Since Nanking 31 is occasionally attacked by smut, the invasion by the hyphae of certain collections of *Ustilago crameri* is of course expected.

## DISCUSSION

It has been known since the work of Dickinson, Hanna, Stakman, and others (11, 12, 13, 19, 23) that segregation of factors for various characters in the smuts occurred either in the first or second division of the fusion nucleus. But there was only circumstantial evidence that reduction in chromosome number had occurred, because the possibility of crossing over could not be eliminated. In the present studies it was demonstrated cytologically for the first time that reduction in chromosome number actually occurred in the first, or the second, or, occasionally, in the third division.

When chlamydospores of *Ustilago crameri* germinate, they produce a promycelium but no sporidia. The nucleus in the chlamydospore apparently is diploid. Meiosis usually occurs within the chlamydospore as it starts to germinate or may occur in the young promycelium. Eventually a 4-celled promycelium is formed, with 1 nucleus in each cell, which, on the basis of behavior and from the standpoint of the number of chromosomes, usually is haploid.

The haploid number of chromosomes is 2, as in most other smuts in which they have been seen, and the diploid number is 4. Occasionally, a diploid nucleus may be included in a promycelial cell. In such cases reduction in chromosome number occurs on the third division of the fusion nucleus. These results furnish cytological proof that some of the unexpected results that have been obtained from isolating sporidia of other smuts may be due to the fact that a promycelial cell may contain a diploid nucleus or 2 haploid nuclei instead of the usual single haploid nucleus.

As no sporidia are formed in *Ustilago crameri,* and the parasitic dicaryophase results principally from fusions between cells of the same promycelium (fusion tubes between different cells or by clamp-like connections between adjacent ones), the chances for recombination of factors for pathogenicity probably are not so great in *Ustilago crameri* as in those smuts that produce sporidia abundantly. However, fusions have been observed between cells of promycelia from different chlamydospores and this may lead to the production of new biotypes. These facts may have an important bearing on the number and constancy of physiologic forms in *Ustilago crameri.*[3]

Penetration into the host usually is accomplished by the promycelium directly, or by branches from it, which evidently are mostly dicaryotic. It appears that penetration is accomplished by mechanical pressure and possibly also by enzymatic action, as the walls of the host seem to be softened

[3] Unpublished data of the writer.

during the process of penetration. Under favorable conditions, the mycelium is well established in the host 3 days after inoculation. The early stages of infection are essentially the same for susceptible and resistant varieties. However, the mycelium rarely reaches the growing point of the resistant varieties; whereas it becomes abundant in that of susceptible varieties within a relatively short time.

SUMMARY

The mature chlamydospore of *Ustilago crameri* has a diploid nucleus, but each promycelial cell usually contains 1 haploid nucleus as a result of reduction division. In the meiotic figures, it is apparent that the chromosome number of the diploid stage is 4 and that of the haploid stage is 2.

Nuclear migration and initiation of the dicaryophase results from knee joints or fusion tubes between adjacent and nonadjacent promycelial cells and between promycelial cells of different chlamydospores. Fusions usually begin immediately after meoisis is completed.

Based on the number of chromosomes and frequency of possible fusion types between promycelial cells, it is concluded that meiosis occurs not only in the first division but frequently in the second division, and occasionally in the third.

The separation of 2 conjugate nuclei that have been associated in a single cell and the subsequent production of uninucleate hyphal cells have been observed.

Binucleate hyphal cells predominate in culture and in the host throughout the life cycle until the late stage of chlamydospore formation. The two haploid nuclei of opposite sex then fuse, and the mature chlamydospore contains only one diploid nucleus.

The process of chlamydospore formation in the host is different from that in artificial cultures. In the host, spores are formed chiefly by segmentation of the hyphal cells, while in culture they usually are formed in an intercalary manner.

The alternation of phases in general is very distinct in *Ustilago crameri;* diplophase, haplophase, and dicaryophase follow in order.

Infections were observed by dicaryotic hyphae in 2-day-old seedlings of resistant and of susceptible varieties when chlamydospores germinated on the host; the promycelia or dicaryotic hyphae derived from them function as infection hyphae and apparently penetrate by mechanical pressure, although there is some evidence of softening of the cell wall also.

In susceptible varieties the infection hyphae develop and branch rapidly and intracellularly in the coleoptile and enter the meristematic tissue as early as 4 days after seed germination, while in the resistant variety only 2 of the 19 seedlings observed were found with hyphae in the meristematic tissues.

UNIVERSITY FARM,
ST. PAUL, MINNESOTA.

## LITERATURE CITED


1. ALLEN, R. F. A cytological study of *Puccinia glumarum* on *Bromus marginatus* and *Triticum vulgare*. Jour. Agr. Res. [U.S.] 36: 487–513. 1928.
2. BAUCH, R. Multipolare Sexualität bei Brandpilzen. Zeits. Induk. Abst. Vererb. 54: 258–259. 1930.
3. BLIZZARD, A. W. The nuclear phenomena and life history of *Urocystis cepulae*. Bull. Torr. Bot. Club 53: 77–117. 1926.
4. BOSS, G. Beiträge zur Zytologie der Ustilagineen. Planta 3: 597–629. 1927.
5. BREFELD, O. Botanische Untersuchungen über Hefenpilze, Heft 5. Die Brandpilze I. 220 pp. Leipzig. 1883.
6. ————. Recent investigations of smut fungi and smut diseases. Trans. from "Nachrichten aus dem Klub der Landwirthe zu Berlin" by E. F. Smith. Jour. Myc. 6: 1–8, 59–71, 153–164. 1890–1891.
7. CHRISTENSEN, C. Haploide Linien von *Ustilago tritici*. Der Züchter 7: 37–39. 1935.
8. CHRISTENSEN, J. J. Studies on the genetics of *Ustilago zeae*. Phytopath. Zeitschr. 4: 129–188. 1931.
9. DANGEARD, P. A. Recherches sur la reproduction sexuelle des champignons. Le Botaniste 3: 221–281. 1893.
10. DICKINSON, S. Experiments on the physiology and genetics of the smut fungi. Hyphal fusions. Proc. Roy. Soc. London Ser. B. 101: 126–136. 1927.
11. ————. Experiments on the physiology and genetics of the smut fungi. Cultural characters. Part 2. The effect of certain external conditions on their segregation. Proc. Roy. Soc. London Ser. B. 108: 395–423. 1931.
12. HANNA, W. F. Studies in the physiology and cytology of *Ustilago zeae* and *Sorosporium reilianum*. Phytopath. 19: 415–442. 1929.
13. HOLTON, C. S. Studies in the genetics and the cytology of *Ustilago avenae* and *Ustilago levis*. Minn. Agr. Exp. Stat. Tech. Bull. 87: 1932.
14. HÜTTIG, W. Ueber den Einfluss der Temperatur auf die Keimung und Geschlechtsverteilung bei Brandpilzen. Zeits. Bot. 24: 529–557. 1931.
15. KNIEP, N. Die Sexualität der niederen Pflanzen. 544 pp. G. Fischer (Jena). 1928.
16. KOLK, LAURA A. Relation of host and pathogen in the oat smut, *Ustilago avenae*. Bull. Torr. Bot. Club 57: 443–507. 1930.
17. LUTMAN, B. F. Some contributions to the life history and cytology of the smuts. Trans. Wisc. Acad. Sci. 16: 1191–1244. 1910.
18. RAWITSCHER, F. Beiträge zur Kenntnis der Ustilagineen I. Zeitschr. Bot. 4: 673–706. 1912.
19. RODENHISER, H. A. Studies on the possible origin of physiologic forms of *Sphacelotheca sorghi* and *S. cruenta*. Jour. Agr. Res. [U.S.] 49: 1069–1086. 1934.
20. SAMPSON, KATHLEEN. The biology of oat smuts III. The development of two biological species of *Ustilago kolleri* (Wille) in a selection of *Avena strigosa orcadensis* (Marquand). Ann. Appl. Biol. 20: 258–271. 1933.
21. SEYFERT, R. Ueber Schnallenbildung im Paarkernmyzel der Brandpilze. Zeitschr. Bot. 19: 577–601. 1927.
22. SLEUMER, H. O. Ueber Sexualität und Zytologie von *Ustilago zeae* (Beckm.) Unger. Zeitschr. Bot. 25: 209–263. 1932.
23. STAKMAN, E. C., J. J. CHRISTENSEN, C. J. EIDE, and BJORN PETURSON. Mutation and hybridization in *Ustilago zeae*. Minn. Agr. Exp. Stat. Tech. Bull. 65: 1929.
24. ————, R. C. CASSELL, and M. B. MOORE. The cytology of *Urocystis occulta*. Phytopath. 24: 874–889. 1934.
25. STEMPELL, K. L. Studien über die Entwicklungsgeschichte einiger Entyloma Arten and über die systematische Stellung der Familie der Sporobolomycetes. Zeitschr. Bot. 28: 225–259. 1935.
26. WALTER, J. M. The mode of entrance of *Ustilago zeae* into corn. Phytopath. 24: 1012–1020. 1934.
27. WANG, C. S. Viability and longevity of chlamydospores of *Ustilago crameri*. Phytopath. 26: 1086–1087. 1936.
28. ————. The formation of chlamydospores of *Ustilago crameri* Kcke. on artificial media. Phytopath. 28: 860–861. 1940.
29. WANG, D. T. Contribution à l'étude des Ustilaginées (Cytologie du parasite et la pathologie de la cellule hôte). Le Botaniste 26: 539–670. 1934.
30. WESTERN, J. H. The biology of oat smuts. IV. The invasion of some susceptible and resistant oat varieties, including Markton, by selected biological species of smut. (*Ustilago avenae* (Pers.) Jens. and *Ustilago kolleri* (Wille)). Ann. Appl. Biol. 23: 245–263. 1936.

# SOME FACTORS INHIBITING THE FRUCTIFICATION AND PRODUCTION OF THE CULTIVATED MUSHROOM, AGARICUS CAMPESTRIS L.

E. O. MADER

(Accepted for publication May 21, 1943)

## INTRODUCTION

It is general knowledge among mushroom growers, particularly those engaged in growing mushrooms in underground caves or mines, that in sections lacking normal air drainage mushrooms of abnormal size and shape are formed. Where there is complete lack of air drainage, fructification ceases entirely. Although there is but little known as to the limiting factors involved, improvement in the air drainage will remedy this condition. Some light was thrown on this subject by the work of Lambert (1) who demonstrated that $CO_2$ in concentrations of five per cent, or more, surrounding the sporophore will cause abnormal growth and, in certain instances, death of the mushroom. No explanation is available up to the present as to the factors causing complete cessation of fructification of mushrooms under conditions where air drainage is at a minimum, and $CO_2$ concentrations are low. In a series of experiments an attempt was made to determine the nature of the substances responsible, or at least to find a way to remove them with methods other than increased air drainage, or circulation. These experiments, a project of the research department of Yoder Bros., were conducted in part in a limestone mine at West Winfield, Pennsylvania, Butler County Mushroom Farms, Inc., and in part at the mushroom plant of Yoder Bros. at Barberton, Ohio.

## EXPERIMENTAL METHODS AND RESULTS OBTAINED

The experiments were conducted in both field and laboratory with the object of applying the results to mushroom culture under mine (cave) conditions. Their primary purpose was the removal of substances interfering with the fructification and production of mushrooms.

The "two-zone" tray system recently described by Lambert (4) was used in these experiments. In essence this system employs individual trays, or growing units, usually not exceeding 10 square feet per tray. The growing media consisted either of horse manure, or of a mixture of horse manure, corn stalks, wheat straw, and protein supplements. The horse manure received 3 to 5 turnings with an average of 17 composting days; the horse manure-organic material mixture, 8 turnings with an average of 25 composting days. Before filling the trays for the individual sub-experiments, the compost was thoroughly mixed so as to reduce the effect of compost heterogeneity on mushroom yields (2, 3). After pasteurization (sweating-out process) the trays were moved to the growing rooms, as needed for the various experiments. For field experiments the average yields of 4 single

10-sq.-ft. trays are recorded for each sub-experiment; (nature of sub-experiment not given). These small individual units were chosen for the reasons described by Lambert (3), who found that "the variance of small plots was in all cases less than the variance of entire beds. Furthermore the small plots permit a greater number of yield comparisons on the same area and also permit increased precision through replication and through the arrangement of the plots to reduce the effect of compost heterogeneity and account for the variability between beds." For laboratory experiments single square-foot units were used.

The compost was planted with a culture of a single-spore strain of the cultivated mushroom *Agaricus campestris* L., received from J. W. Sinden of the Pennsylvania State College. When the mycelium growth was complete, the trays were cased with soil for fructification and production.

Other material, as well as the methods employed, are described under each series of experiments.

## Series 1. The Effect of "Locations within the Mine" on the Fructification and Production of Mushrooms

Duplicate trays of several experiments were set in different rooms of the mine in order to study the effects of "locations within the mine" on fructification and production of mushrooms. For all experiments, except 33, the mycelium was grown and fructified in the same location. In experiment 33, part of the trays, after the mycelium growth was complete, were moved to other locations for fructification.

TABLE 1.—*The effect of "locations within the mine" on mushroom production*

| Mycelium grown | Mushroom produced | Number of days picked | Average yields in lb. per pounds per sq. ft. |
|---|---|---|---|
| Experiment 18. Duration: December, 1937, to July, 1938 | | | |
| Mine room B | Mine room B | 120 | 2.69 |
| Mine room 53 | Mine room 53 | 120 | 0.55 |
| Experiment 24. Duration: January, 1938, to August, 1938 | | | |
| Mine room B | Mine room B | 120 | 1.75 |
| Mine room 53 | Mine room 53 | 120 | 0.94 |
| Experiment 33. Duration: March, 1938, to October, 1938 | | | |
| Mine room B | Mine room B | 84 | 2.48 |
| Mine room B | Mine room C–R | 84 | 1.93 |
| Mine room B | Mine room 94 | 84 | 1.81 |

*Summary of Facts and Conclusions of Experiments Under Series 1.* During the experiment it became evident that the various locations within the mine affected mushroom yields. Trays in room B consistently outyielded those in other rooms. This room, being close to the main air passage of the mine, received excellent air drainage. The air movement of the other rooms was subject to whatever air exchange was present for the

entire mine throughout the time the experiments were conducted. This air exchange varied with the prevailing atmospheric conditions outside the mine. These rooms, because of their locations within the mine, were never at optimum conditions for mushroom production.

Numerous tests for carbon monoxide and carbon dioxide were made at short intervals for the various locations in the mine. Although it was possible that the exhaust of the motor vehicles used, as well as the fumes from the workers' lamps, might have increased the monoxide content of the mine atmosphere, no trace of this gas was found in any of the samples taken. The carbon dioxide content of the air varied, but never exceeded 0.06 per cent in any sample taken. The temperature of the mine (13 to 14° C.) and the humidity (95 to 97 per cent) were constant throughout the duration of these investigations. The differences in yields obtained for the various rooms could hardly be explained by the above-mentioned factors.

It was of interest to note that, particularly at the beginning of fructification, the trays were covered with an abundance of small sporophores. Trays in room 53 not only showed the largest number of sporophores, but also strands of heavy vegetative mycelium. Quite a number of these small sporophores did not develop into mushrooms and died off. Trays in room B showed normal fructification without the heavy mycelium strand formation.

Since such a proliferous tendency to fructification is seldom found in mushroom houses, this phenomenon suggests the presence of a substance, or substances, in the mine that, in small amounts, act as a stimulant to fructification. When present in larger amounts it will not only arrest the sporophore growth, but will entirely prevent fructification.

Since the mushroom mine may be considered as a large incubator with constant humidity and temperature and limited natural air drainage, the behavior of the mushroom (or sporophore) suggests that the effect of these toxic substances is general. This assumption led to two sets of experiments that will be described as the "closed-room-effect" and the "place-effect" experiments.

The term "closed-room-effect" designates a condition in which the air circulation is kept at a minimum, allowing for the accumulation of toxic substances in such quantities as to inhibit entirely the fructification and production of the mushroom.

The term "place-effect" designates the condition of growing mushrooms in both mine and mushroom house and measuring the difference in yields in order to establish a relative depressing effect of the mine at any given time.

## Series 2. The "Place Effect" on the Fructification of Mushrooms

From the numerous experiments in this series conducted from 1938 to 1943, inclusive, only 4 will be presented here to demonstrate the fluctuation of the mine atmosphere and its effect on mushroom fructification and pro-

duction as compared with house atmosphere. In these experiments notes also were taken on the development of mycelium under these conditions. For comparative tests identical sets of trays were grown in the mine and the mushroom house. The mushroom house located at Barberton, Ohio, will be designated as "house," and the limestone mine (cave) located at West Winfield, Pa., as "mine" (Table 2).

After completion of the growth of the mycelium, halves of either set of trays were interchanged in order to study the "place effect" on the fructification and production of mushrooms.

TABLE 2.—*The place effect of house and mine culture on the production of mushrooms*

| Mycelium grown | Mushrooms produced | Number of days picked | Pounds per sq. ft. sub-experiment | | | | |
|---|---|---|---|---|---|---|---|
| Experiment R–9B. Duration: Oct., 1938, to May, 1939 | | | | | | | |
| | | | 1 | 2 | 3 | 4 | 5 |
| House | House | 94 | 1.41 | 1.57 | 1.76 | 1.66 | 1.88 |
| House | Mine | 94 | 1.45 | 1.71 | 1.92 | 1.83 | 2.12 |
| Experiment EE. Duration: Oct., 1940, to July, 1941 | | | | | | | |
| | | | EE–1 | EE–2 | EE–3 | EE–4 | EE–5 |
| Mine | Mine | 106 | 1.30 | 0.80 | 1.12 | 1.51 | 1.67 |
| Mine | House | 61 | 1.71 | 1.29 | 1.65 | 2.32 | 2.38 |
| House | House | 128 | 2.42 | 1.62 | 2.40 | 2.91 | 2.55 |
| House | Mine | 125 | 1.87 | 1.51 | 2.03 | 2.42 | 2.17 |
| Experiment A–B. Duration: Oct., 1941, to May, 1942 | | | | | | | |
| | | | 6 | 7 | 13 | 15 | 16 |
| House | House | 80 | 1.87 | 2.29 | 2.11 | 2.33 | 2.16 |
| House | Mine | 80 | 1.49 | 1.84 | 1.51 | 1.72 | 1.51 |
| Experiment 1A, 1B, 3A. Duration: Oct., 1942, to March, 1943 | | | | | | | |
| | | | 1A | 1B | 3A | | |
| House | House | 94 | 2.01 | 2.16 | 1.81 | | |
| House | Mine | 84 | 1.86 | 2.21 | 1.85 | | |
| Mine | Mine | 67 | 1.88 | 2.07 | 1.97 | | |

*Summary of Facts and Conclusion of Experiments Under Series 2.* Mycelium, grown under mine or house conditions, was excellent. There was no visible "place effect" on its development.

Conditions under which fructification and production of mushrooms took place varied. Since the trays of experiment R–9B were placed directly in the main air passages of the mine, the constant natural air flow present was sufficient to prevent accumulation of substances interfering with the fructification and production of the mushrooms. In this experiment no place effect of the mine could be observed. However, trays placed in other sec-

tions of the mine, but not included in this experiment, showed a distinct place effect, that is, lower yields resulted.

Shortly before experiment 1A, 1B, and 3A was started, an artificial ventilation system was installed in the mine. Inasmuch as the mine yields obtained in this experiment equalled house yields, it may be concluded that the artificial ventilation of the mine was sufficient to remove any harmful gases present.

The results of the two other experiments (EE and A–B) reflect the unfavorable atmospheric conditions of the mine. During the experimental periods there was little actual air movement in the mine, and the accumulation of toxic substances must have been considerable. Increased sporophore formation, dying of small mushrooms, small-sized mushrooms and lower production yields resulted as compared with those produced under house conditions during the same period.

These experiments have failed to show why mine mushrooms are smaller on the average than house mushrooms. The causes for this phenomenon are not necessarily associated with production yields. Production in the mine was equal to house production, but the mine mushrooms were smaller in size than the house mushrooms (Table 2, Exp. R–9B and 1A, 1B, 3A).

## Series 3. The Closed-room Effect on the Fructification and Production of Mushrooms

Experiments in Series 1 and 2 demonstrated that mine rooms having but little air movement exerted a harmful effect on the fructification and production of mushrooms. The question naturally arises as to what would happen if the air circulation in the mine room was cut off entirely.

The dead ends of two mine rooms were sealed off with building paper from the remainder of the rooms. No attempt was made to have them absolutely air-tight. The sealed-off sections were labeled "inside" and the remaining rooms were marked "outside," and had access to the normal air drainage of the mine. Trays with previously prepared compost were set up in each section, and the mycelium was grown both "inside" and "out-

TABLE 3.—*The effect of "inside" and "outside" of mine rooms on fructification and production of mushrooms*

(Duration of the experiment: Dec., 1938, to Aug., 1939)[a]

| Mycelium grown | Mushrooms produced | Lb. per sq. ft. |
|---|---|---|
| Inside room 54 | Inside room 54 | 0.00 |
| Inside room 54 | Outside room 54 | 0.77 |
| Outside room 54 | Inside room 54 | 0.00 |
| Outside room 54 | Outside room 54 | 1.32 |
| Inside room D | Inside room D | 0.00 |
| Inside room D | Outside room D | 0.77 |
| Outside room D | Inside room D | 0.02 |
| Outside room D | Outside room D | 1.48 |

[a] Dr. E. F. Hopkins of the Yoder Bros. Research Department was in charge of this experiment at West Winfield, Pa.

side." The other halves of either section of the rooms remained where they were grown.

*Summary of Facts and Conclusion of Experiment under Series 3.* From its appearance the mycelium grown both "inside" and "outside" appeared excellent.

Fructification, however, took place only in the "outside" sections of the rooms; and this occurred irrespective of where the mycelium was grown. There was one exception, however. Mycelium grown "outside" room D and then moved "inside" the same room produced some mushrooms. The reason for this apparent contradiction of the closed-room effect on fructification was found in later studies given under Series 4. Here it was demonstrated that when sporophore formation took place before the trays were exposed to the closed-room effect some of the mushrooms developed. This undoubtedly took place with the mycelium grown "outside" room D, since, as has been frequently observed, mycelium grown under mine conditions will fructify, even in absence of casing soil. Undoubtedly some of the mycelium in this group of trays fructified before it was cased and placed "inside." Hence the mushroom yields recorded.

Mycelium grown "inside" and then moved "outside" for fructification produced only half as much as that grown and left "outside" for production. This would indicate that mycelium grown under closed-room conditions (inside) has a lower production capacity.

The mine with its limited natural air flow can be considered a semi-closed room, and mycelium grown under such conditions should have a production capacity inferior to that grown under house conditions. This actually has been demonstrated. As will be recalled (Experiment EE, Table 2) mycelium grown under mine conditions resulted in inferior yields when compared with that from house conditions. When there is a positive and continuous air movement (artificial ventilation) mycelium grown under mine conditions will produce equally as well as that grown under house conditions (experiment 1A, 1B, 3A).

## Series 4. The Fructification of Mushrooms under Controlled Atmospheric Conditions

In the preceding experiments it has been shown, that in mines (caves) lacking air drainage or exchange, mushroom fructification and production will be affected. Questions arise as to the nature of the substances responsible and the possibility of removing them.

It is general knowledge that waste products formed in the metabolic processes of fungi exert a harmful effect on the producing organism. The term staling products has been applied because the nature of these products is not fully understood. Similar substances are produced by the microflora responsible for the decomposition (conditioning) of the organic materials used in the preparation of the mushroom compost. An accumulation of these products continues in this medium, coming in part from the microflora

present, and in part from the cultivated mushroom itself. In commercial mushroom culture it is impossible to remove these substances from the growing medium either by washing or leaching. Additions of chemicals such as copper sulphate to the growing medium or the improvement of its oxidation-reduction system through the use of manganese sulphate and ferric sulphate resulted in increased yields (unpublished data). Aside from those that combined with the medium itself, there are some that, due to their volatile nature, are released into the surrounding atmosphere. These only will be considered in this paper.

It has been shown that air polluted with these volatile substances will be detrimental to the fructification and production of mushrooms. Under normal growing conditions, such as prevail in mushroom houses, these substances are readily removed and carried off with an exchange of air. Furthermore, the fluctuation of temperature (and humidity) in the houses and its effect on the periodic drying of the casing soil and the culture medium, will greatly facilitate the removal of these gases.

Due to the constant temperature and humidity conditions in the mushroom mine, it can be assumed that the rate of liberation of these substances from the growing media is slow. Such an accumulation in the medium will affect fructification and production of mushrooms. If present in small amounts, such substances may stimulate vegetative growth. It has been observed generally that mycelium grown under mine conditions is more profuse than that grown under house conditions. The proliferous sporophore formation may also fall under such a stimulant effect. It is an established fact that such a proliferous sporophore formation usually results in a crop of small mushrooms, particularly in mines. High accumulations of these substances in the culture medium, as well as in the surrounding atmosphere, will stop fructification entirely. The term "closed-room effect" has been applied to such conditions (Series 2).

The removal of these substances by artificial air exchange, a problem of mechanical nature, has not been considered in these studies. Due to the volatile nature of these substances the following experiments were designed to remove them by washing the air.

## EXPERIMENTAL METHOD AND PROCEDURE

To simulate cave, or closed-room conditions, special chambers for growing mushrooms were built. The frames were made of wood and painted with du Pont primer. The sides were of glass. One end of the chamber was removable, and after it was loaded with the mushroom trays, it was sealed with putty. There was an outlet on each end for air-tube connections. These chambers had a capacity of 12 cu. ft. each, and were set in a large room in which the average temperature was 14° C. Small air pumps, 2 cu. ft. capacity per minute, were used for circulating the air in the mushroom chambers. The air was pumped through the various air-washing equipment periodically. Water traps were installed between the solutions

used, except in the sulphuric acid experiment, where the desired relative humidity in the culture chambers was maintained. The following materials for washing the air were used: 1. Alkaline potassium permanganate solution 3 lb. of sodium hydroxide plus 2 ounces of potassium permanganate per 5 gal. of water. 2. Dorex Cannister containing activated charcoal, obtained from the W. B. Connor Engineering Co., New York, N. Y. 3. Mineral oil (commercial grade). 4. Sulphuric acid (commercial grade).

The mycelium for these experiments was grown in special sq. ft. trays. After completion of the growth, the trays were cased with soil, watered, and placed in the various chambers. After loading the chambers, the outlets were connected with the air-washing equipment by means of rubber tubing.

*Duration of the experiment:* August, 1942, to March, 1943.

## Experiment A

In this set the mycelium was grown under house conditions. After the trays were cased with soil they were placed in the chambers. The air in chambers 1 and 2 was washed periodically at 6-hour intervals, moving 40 cu. ft. of air through the washing units. Chamber 3 did not receive any washing.

*Chamber 1.* Direction of air flow: mushroom chamber—airpump—water trap—mineral oil—water trap—alkaline potassium permanganate solution—water trap—return to mushroom chamber. *Results.* Sporophores formed after 17 days of washing. The development of the fruit bodies was normal.

*Chamber 2.* Direction of air flow: mushroom chamber—airpump—Dorex Cannister—return to mushroom chamber. *Results.* Sporophores formed after 19 days of washing. The development of the fruit bodies was normal.

*Chamber 3.* No washing of the air. *Results.* A heavy surface growth of the mycelium appeared on top of the casing soil. No sporophore formation occurred.

## Experiment B

As in experiment A, mycelium that had completed its growth was used in this set. Here, an attempt was made to differentiate between the ability of mineral oil and that of alkaline potassium permanganate solution to remove the substances. The same period for washing the air was maintained as in experiment A.

*Chamber 1.* Direction of air flow: mushroom chamber—airpump—water trap—alkaline potassium permanganate solution—water trap—return to mushroom chamber. *Results.* Sporophores formed after 16 days of washing. The development of the fruit bodies was normal.

*Chamber 2.* Direction of air flow: mushroom chamber—airpump—water trap—mineral oil—water trap—return to mushroom chamber. *Results.* Sporophores formed after 16 days of washing. The developing fruit bodies showed the typical $CO_2$ effect, as described by Lambert (1).

The stipe was elongated and the pileus was retarded in its development, giving the mushroom an out-of-proportion appearance.

*Chamber 3.* No washing of the air. *Results.* Similar to those as given under chamber 3, experiment A.

## Experiment C

In this set an attempt was made to determine firstly (tray *a*), whether trays in the process of fructification and production would continue if placed under closed-room conditions; secondly (tray *b*), whether cased mycelium exposed to closed-room conditions for a period of 28 days could be made to fructify and produce mushrooms; thirdly (tray *c*), the use of outside-grown mycelium for comparison.

After loading the trays in the chamber, the air in chambers 1 and 2 was washed for the same interval as given under experiments A and B.

*Chamber 1 and 2* (duplicates). Direction of air flow: mushroom chamber—airpump—water trap—alkaline potassium permanganate solution—water trap—return to mushroom chamber.

*Tray a. Results.* Sporophores continued to appear. Fruit bodies present before trays were put into chamber continued to develop normally.

*Tray b. Results.* Sporophores developed after 26 days of washing. The total number formed was somewhat less than those forming on tray *c*. The development of the fruit bodies was normal.

*Tray c. Results.* Sporophores formed after 16 days of washing. The development of the fruit bodies was normal.

*Chamber 3.* No washing of the air.

*Tray a. Results.* No further sporophores formed. Some of the fruit bodies present developed into mushrooms of gigantic proportions. The length of stipe was about 2 to 3 times that of outside-grown mushrooms. It was swollen at the base and of dumbbell shape. The pileus was abnormally large, misshapen, and not so symmetrical as that of a normal mushroom. Some fruit bodies had a small pileus resembling those formed under conditions having a high $CO_2$ concentration of atmosphere. The rate of development of these fruit bodies was very rapid, and at maturity they soon collapsed. Other fruit bodies remained "seated." They bulged, onion-like, at the base.

*Tray b and c. Results.* No sporophore formation. There was a heavy growth of mycelium on top of the casing soil.

## Experiment D

In this set an attempt was made to determine whether the relative humidity of the air surrounding the mushroom trays affects sporophore formation and the development of the fruit bodies. Sulphuric acid served as the humidity-control agent. The concentrations of the acid were determined by specific-gravity measurements (5, 6). The alkaline potassium permanganate solution was employed for removal of the volatile gases. The air in

the chambers was washed at 2-hour intervals over a period of 28 days. The sulphuric acid was replaced every 4 days through the period of the experiment. Mycelium grown under outside conditions was used after completion of its growth.

*Chamber 1.* Direction of air flow: mushroom chamber—airpump—water trap—alkaline potassium permanganate solution—water trap—sulphuric acid at a concentration to maintain an average humidity of 35 per cent—return to mushroom chamber. *Results.* Sporophores formed after 18 days of washing. The developing fruit bodies were normal in shape, except for their size. It was observed that these mushrooms were more compact and had a shorter stipe and smaller pileus than mushrooms grown under outside conditions.

*Chamber 2.* Direction of air flow: mushroom chamber—airpump—water trap—alkaline potassium permanganate solution—water trap—sulphuric acid at a concentration to maintain an average humidity of 88 per cent—return to mushroom chamber. *Results.* Sporophores formed after washing 19 days. The developing fruit bodies were of normal shape and size.

*Summary of Facts and Conclusions of Experiments under Series 4.* Trays placed in air-tight chambers receiving no air washing failed to fructify. Conditions in these chambers did not interfere with the growth of the mycelium. A heavy surface growth on top of the casing soil was present on all of the trays exposed to these conditions.

Trays in the process of production placed in these chambers exhibited an interesting picture. No new sporophore formation took place. Fruit bodies present before the trays were placed in the chambers continued to develop. Some of them grew into mushrooms of abnormal shape and size, others remained "seated," bulging, onion-like, only at the base of the stipe. The large fruit bodies formed collapsed soon after reaching maturity and disintegrated. Their keeping quality was poor in comparison with outside-grown mushrooms. Sister trays, placed in chambers from which the toxic substances were removed, continued to fructify and develop normal mushrooms.

Removal of the toxic substances inhibiting fructification was accomplished with either mineral oil, alkaline potassium permanganate solution, or activated charcoal. Since mineral oil did not remove the $CO_2$, an accumulation of this gas in the chamber resulted in malformed mushrooms, as described by Lambert (1). The fact that these substances can be removed with mineral oil suggests that their nature may be that of unsaturated hydrocarbons, readily soluble in a highly purified hydrocarbon. Solutions of alkaline permanganate removed the toxic substances by oxidation. This implies that these toxic substances may be of the nature of non-saturated hydrocarbons, which are readily oxidized.

The reason for the common practice of supplying air to growing mushrooms can be explained on this basis. Positive air movement insures a bet-

ter oxygen supply, particularly in regions where the mycelium grows. Under mushroom-house conditions, this is easily accomplished. The frequent watering and drying of the mushroom media will aid this process; hence, normal mushroom yields in houses. Under mine conditions, due to constant temperature and humidity, there is a minimum of air movement, which, in part, explains the lower mushroom yields obtained under these conditions.

Activated charcoal, the principal ingredient of the Dorex Cannister, was also effective in removing the toxic substances, together with the $CO_2$ present.

Whether the humidity was maintained at either 35 or 88 per cent was immaterial for sporophore formation in these experiments. Low humidity, however, restricted the development of the fruit body, giving mushrooms of normal shape but smaller than those formed in higher humidity.

It was of interest to note that mycelium previously exposed to closed-room conditions over a period of 28 days after casing could be made to fructify by washing the air. The mycelium, however, needed a longer time for sporophore formation than normally cased mycelium. Whether this delay in fructification was due to an exposure of the mycelium to closed-room conditions after casing, or to a weakening of the mycelium by prolonged respiration has not been determined.

The very fact that substances belonging to the unsaturated hydrocarbons are effective in inhibiting and preventing fructification of the cultivated mushroom *Agaricus campestris* without interfering with the growth of the mycelium proper, as shown in this paper, suggests that: Substances of the same nature could be used effectively in the control of some plant diseases caused by fungi. Preventing the fungus from fructifying eliminates dissemination of spores and the disease will be restricted to its primary infection centers, even though the mycelium may continue to grow unchecked vegetatively as these substances may not have fungicidal value in the accepted terms.

## SUMMARY

Conditions affecting mushroom fructification and production have been investigated.

It was found that wherever mushrooms are grown, substances of volatile nature accumulate and become detrimental to their growth and fructification.

Under mine conditions certain rooms tend to accumulate these substances more readily than others, and the term "locations within the mine" has been applied to this phenomenon.

In mushroom houses high yields are produced as a result of constant mechanical ventilation, whereas in mines where the air exchange is dependent upon natural ventilation, yields are lower. The term place effect describes these conditions. Improving the ventilation in the mine will raise yields to the production level of the mushroom house.

When these substances were permitted to accumulate, fructification

ceased entirely. This was accomplished by sealing up mine rooms or experimental chambers (closed-room effect).

The effect of these substances on mushrooms varied. Mycelium grown where these substances were allowed to accumulate had a lower production capacity than that grown where they were removed. It was impossible, visibly, to distinguish between mycelium grown under either conditions. Under mine conditions where there was but a partial removal of these substances, the effect on fruit bodies was observed in various stages ranging from increased fructification (sporophore formation) to complete cessation of fructification. Sporophores exposed to conditions allowing for an accumulation of these substances either developed into fruit bodies of gigantic size and abnormal shape, or they did not elongate at all and grew only at the base of the stipe, assuming an onion-like appearance.

Removal of these substances has been accomplished by washing the atmosphere. Alkaline potassium permanganate solutions, mineral oil, and activated charcoal proved to be effective agents for this purpose.

The nature of these substances is unknown. However, the chemicals successfully used for their removal suggest that they belong to the class of nonsaturated hydrocarbons.

Their origin has not been determined, but it is assumed that they are products of the metabolism of the mushrooms themselves, or of the microflora present in the growing media.

Humidity control of the atmosphere surrounding the trays did not affect sporophore formation.

DOYLESTOWN, OHIO.

LITERATURE CITED

1. LAMBERT, E. B. Effect of excess carbon dioxide on growing mushrooms. Jour. Agr. Res. [U.S.] 47: 599–608. 1933.
2. ————, and A. C. DAVIS. Distribution of oxygen and carbon dioxide in mushroom compost heaps as affecting microbial thermogenesis, acidity, and moisture therein. Jour. Agr. Res. [U.S.] 48: 587–601. 1934.
3. ————. Size and arrangement of plots for yield tests with cultivated mushrooms. Jour. Agr. Res. [U.S.] 48: 971–980. 1934.
4. ————. Mushroom growing in the United States. U. S. Dept. Agr. Farmers Bull. 1875: 1–37. 1941.
5. STEVENS, NEIL E. A method for studying the humidity relations of fungi in culture. Phytopath. 6: 428–432. 1926.
6. WILSON, ROBERT E. Humidity control by means of sulphuric acid solution with critical compilation of vapor pressure data. Jour. Indust. and Eng. Chem. 13: 326–331. 1921.

# THE FUNCTION OF LIME AND HOST LEAVES IN THE ACTION OF BORDEAUX MIXTURE

C. E. YARWOOD

(Accepted for publication April 29, 1943)

The importance of lime in Bordeaux mixture may be emphasized by the fact that, although the fungicidal value of copper sulphate was reported by Prévost in 1807 (10), it was not until lime was added to the bluestone solution that a protective copper fungicide suitable to foliage was developed by Millardet, and reported in detail in 1885 (9). Millardet found that 0.01 per cent lime or 0.000025 per cent bluestone would inhibit the germination of the sporangia of *Plasmopara viticola,* but the spray he recommended for field control contained 1190 times the above content of lime and 238,000 times the above content of copper, indicating that Millardet was possibly aware of some great apparent discrepancy between *in vitro* and *in vivo* values of the fungicidal value of copper. According to Millardet (9), "The lime seems to me, then, to play a triple role in the mixture. At the moment of spraying it acts as an energetic mordant, which fixes the disinfecting drop on the leaf and establishes its close adherence. For several days, it is capable of killing the conidia and zoospores by its causticity. Finally, when it has been transformed into carbonate, it serves for the preservation of the store of copper oxide." It is this last function of lime listed by Millardet, but which might be stated differently, that the writer considers most important, and that will be treated in this paper. Another important aspect of the function of lime, not considered by Millardet, and not treated in this paper, is the injurious effect of lime on the host, as discussed by Horsfall and Suit (6).

Several investigators have indicated differences between the apparent *in vitro* and *in vivo* toxicity of copper sprays. Marsh (8) reported that conidia of *Venturia inaequalis* did not germinate on slides sprayed with copper cyanide, but germinated to the extent of 39 per cent on leaves to which the same dosage of copper cyanide had been applied. Howard (7) showed that several samples of Bordeaux were more toxic to *Alternaria solani* conidia on glass slides than on potato leaves. Something in the leaves apparently reduced the toxicity of the copper. It has been shown by Clark (2) and confirmed by the writer (12) that asparagin, a normal constituent of leaves, may greatly reduce the toxicity of copper sulphate or Bordeaux in *in vitro* tests. The literature on antagonism in relation to copper is quite extensive, and will not be reviewed here.

## METHODS

The dosage-response technique of Wilcoxon and McCallan (11) was used in this study, and the fungicidal value of lime alone, of bluestone alone, and of mixtures of bluestone and lime were measured throughout a series of

dosages on glass and leaf surfaces. The dosage increment (ratio of concentration of successive dosages) of 3 generally used in this study, is more than that used by Wilcoxon and McCallan and is probably too great for careful studies of such aspects as changes in slope of the dosage response curve, but was considered adequate for purposes of this study. Dosages were chosen to determine principally the LD 50, LD 95, and intermediate values, to the considerable neglect of values below LD 50. The straight line best fitting the points in the region of LD 50 to LD 95 is taken as the dosage response curve. Many experiments gave approximately straight line curves, but a more detailed study might show these curves to be other than straight lines.

Bordeaux mixture was prepared by adding equal amounts of 10 per cent bluestone ($CuSO_4 \cdot 5H_2O$) and 10 per cent lime ($CaO$) to the required amount of water, unless otherwise mentioned. The stated concentrations of Bordeaux are the concentrations of bluestone in this equal lime-Bordeaux mixture.

Bean rust, cucumber downy mildew, and powdery mildew of bean were the diseases studied in the greenhouse and laboratory experiments to which this report is confined. Bean rust (*Uromyces phaseoli* (Pers.) Wint.) was grown on the primary true leaves of potted Pinto beans. To secure spores, infected leaves were sprayed with tap water by means of an atomizer, and the suspension of urediospores secured in this way was sprayed immediately onto the surfaces bearing the dried test spray deposit—Syracuse watch glasses for germination tests, leaf surfaces for protection tests. The concentration of spores was not determined, but was such as to give from 2 to 25 spores per sq. mm. in germination tests and 40 to 125 pustules per sq. cm. on control leaves in protection tests. Inoculated plants were held overnight in a moist chamber and then left on the greenhouse bench until infection was measured by counting the uredinial pustules.

Cucumber downy mildew (*Pseudoperonospora cubensis* (B. and C.) Rostew.) was grown on the secondary leaves of potted cucumber plants, variety Long Green. To secure sporangia, leaves that had been infected 5 to 10 days were incubated overnight in moist chambers, and the sporangia formed were washed off to form a water suspension, used to inoculate sprayed and control plants in protection tests and to seed onto spray deposits on glass. The spore dosage was such as to give about 5 sporangia per sq. mm. germination tests and about 20 downy mildew lesions per sq. in. on control leaves in protection tests.

In protection tests on bean and cucumber foliage a range of dosages was secured by using sprays of different fungicide content and spraying to the run-off stage with a compressed-air atomizer. The initial wet deposit per sq. dm. of leaf surface secured in this way without a spreader was variable, but averaged about 1 g. for the lower surface of bean leaves, 0.8 g. for the upper surface of bean leaves, 2 g. for the lower surface of cucumber leaves and 1.5 g. for the upper surface of cucumber leaves. Sprays without supplements usually gave fair coverage on beans but poor on cucumbers. When

0.05 per cent glyceryl phthalic alkyd resin was added as a spreader to the sprays the deposit per sq. dm. was about 0.6 g. for the upper or lower surfaces of bean leaves, 1 g. for the lower surface of cucumber leaves and 0.7 g. for the upper surface of cucumber leaves. The amount of fungicide per unit area was calculated from the above figures on wet deposit and from the known fungicide content of the spray. For infection counts in protection tests the number of rust pustules in 3 to 8 (depending on variability and amount of infection) sq. cm. areas was counted for each treatment, and these values were converted to per cent reduction in number of rust pustules (= per cent control) by expressing the difference in the number of pustules per unit area for treated and control (= check) leaves as a percentage of the number of pustules on the control leaves. For infection counts of cucumber downy mildew the number of lesions on 2 to 4 sq.-in. areas was counted for each treatment and the per cent of control was determined in the same way as for bean rust.

Powdery mildew of the bean (*Erysiphe polygoni* DC.), grown on the primary leaves of Pinto beans, was employed in a study of the eradicant action of fungicides. Bean plants were inoculated by spraying the leaves on both surfaces with a suspension of conidia secured from infected plants. At 4 to 6 days after inoculation, when infection was just visible, the inoculated plants were sprayed with the test spray containing 0.05 per cent phthalic glyceryl alkyd resin as a wetting agent in addition to the test dosages of bluestone or lime. The wetting agent was added because it increased the uniformity of the results and increased the effectiveness of most sprays, but had very little effect when used alone at the low impact pressures used in these tests. The choice of 4 to 6 days as the interval from inoculation to the use of eradicant sprays is arbitrary but it is important to bear in mind that the resistance of powdery mildew colonies to killing by eradicant sprays increases with the age of the colonies (data not presented here). At about 5 days after spraying (9 to 11 days after inoculation) the amount of mildew was estimated on an arbitrary scale of 0 to 10 in which 0 indicated no mildew present and 10 that the leaf surface was entirely covered. The upper and lower leaf surfaces were rated independently. Per cent control was calculated by expressing the difference in rating between treated and control leaves as a percentage of the rating for the control.

In studies of the effect of copper sulphate and lime on germination, 3 cc. of the test spray was added with a pipette to Syracuse watch glasses of 51 mm. diameter and allowed to dry. This gave a uniform deposit of fungicide of known dosage onto which the spore suspension was later sprayed. The cultures were incubated at 16° C. in the dark for 24 hours and the germination of 100 spores per dish for each test was determined. The presence of a germ tube was the criterion of germination in the case of bean-rust urediospores, and the absence of contents and papilla was the criterion of germination for the sporangia of cucumber mildew. The heavy deposit of lime required to inhibit germination of bean-rust urediospores and downy-mildew

sporangia, and the heavy deposit of Bordeaux required to inhibit germination of bean-rust urediospores made observations of germination in these *in vitro* cultures very difficult and the results rather inaccurate.

### PHYTOCIDAL ACTION OF SPRAY MIXTURES

In order to evaluate properly the effect of a fungicide in controlling a fungus disease it is desirable to know the effect of the fungicide on the host in the absence of the disease. As is well known, bluestone is more toxic to foliage than lime or Bordeaux mixture at similar concentrations. Results of one comparative test on beans and cucumbers are given in figure 1. In this test plants with secondary leaves were sprayed with the test mixtures on September 2, 1942, and the living tissues of the leaves were weighed on September 7. Bluestone at 0.1 per cent caused about 5 per cent injury and

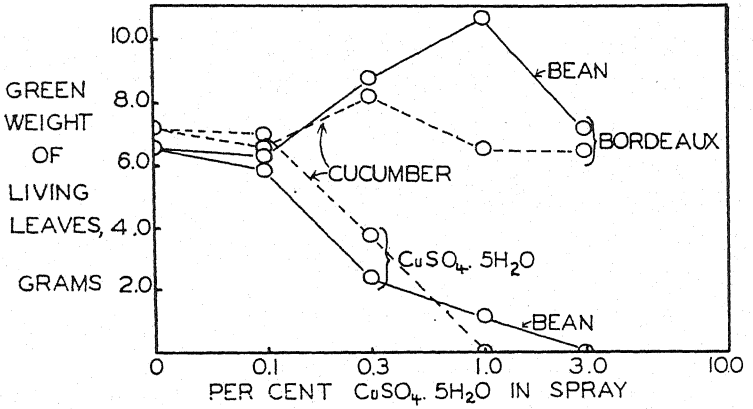



FIG. 1. The phytocidal effect of bluestone and Bordeaux on bean and cucumber foliage.

3 per cent bluestone caused complete killing of the bean and cucumber foliage, while 3 per cent Bordeaux did not cause marked injury. Lime alone was not used in this test, but in other tests lime has caused no apparent injury at 1 per cent.

### PROTECTIVE ACTION ON LEAVES AND TOXICITY ON GLASS

Results of two tests of the protective action of bluestone, lime, and Bordeaux for bean rust are summarized graphically in figure 2. Here the concentration of the spray applied to the leaves is plotted against per cent reduction in number of rust pustules (= per cent control). These results are representative of the variation between tests, and of the deviation from straight lines of the dosage response curves plotted in this manner. In order to render results of this type more closely comparable to those on glass surfaces, all results were converted to a dosage-per-unit-area basis. Average LD 50 and LD 95 values for bean rust of all comparative tests of the effect of bluestone, lime, and Bordeaux on the inhibition of germination on glass and the protection from infection on leaves are presented in table 1.

Several important findings are indicated from the data of table 1. To obtain 95 per cent inhibition of germination of bean-rust urediospores on glass required more than 100 times as much copper in the form of Bordeaux as in that of bluestone, indicating that lime decreased the fungicidal action of the copper on glass surfaces. On the other hand, to obtain 95 per cent control on leaves required more than 10 times as much copper in the form of bluestone as in the form of Bordeaux, indicating that lime enhanced the fungicidal value of the copper on leaves. To obtain 95 per cent reduction in germination on glass required 350 times as much Bordeaux mixture as to obtain 95 per cent control on leaves, further emphasizing the important difference between glass and leaves as a substrate for the evaluation of fungicidal action.

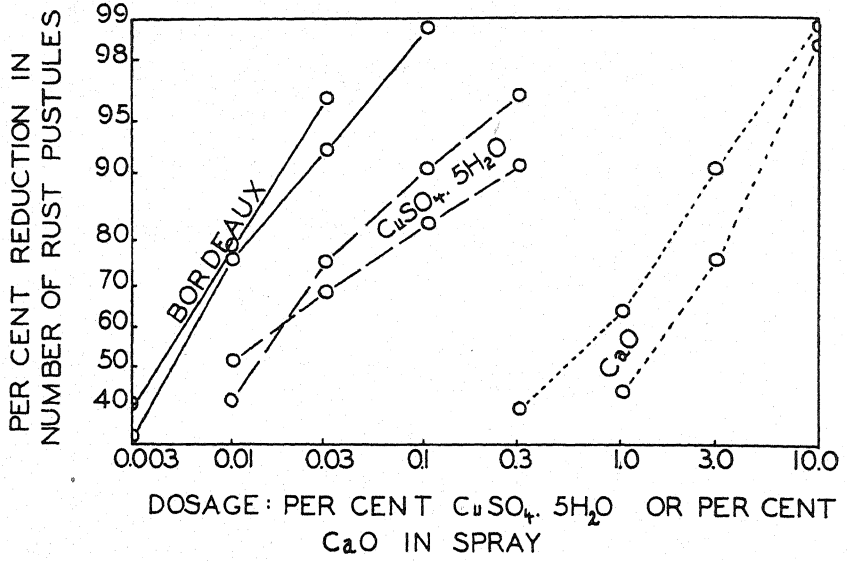



FIG. 2. Relation between spray dosage and protection (reduction in number of uredinial pustules) from bean rust.

TABLE 1.—*Comparative toxicity and protective value of lime, bluestone, and Bordeaux mixture for bean rust*

| Fungicide | Germination on glass.[a] Dosage per square decimeter for | | Protection on plants.[b] Dosage per square decimeter for | |
|---|---|---|---|---|
| | 50 per cent reduction in germination | 95 per cent reduction in germination | 50 per cent reduction in number of rust pustules | 95 per cent reduction in number of rust pustules |
| CaO | 60 mg. CaO | 300 mg. CaO | 28 mg. CaO | 124 mg. CaO |
| $CuSO_4 \cdot 5H_2O$ | 0.113 mg. Cu | 0.60 mg. Cu | 0.062 mg. Cu | 2.1 mg. Cu |
| Bordeaux | 15 mg. Cu | 64 mg. Cu | 0.022 mg. Cu | 0.18 mg. Cu |

[a] Average of 6 tests for CaO, 9 for $CuSO_4 \cdot 5H_2O$, and 11 for Bordeaux.
[b] Average of 3 tests for CaO, 8 for $CuSO_4 \cdot 5H_2O$, and 8 for Bordeaux.

A source of error not allowed for in these tests was the greater spore dosage per unit area in the toxicity tests on glass than in the protective tests

on leaves. The spore dosage of the inoculum on leaves was not determined, and, since infection results were recorded as pustules per sq. cm., the writer does not know with certainty how spore-dosage data could be made use of here, even though they were known.

Studies of the comparative toxicity and protective values of lime, bluestone, and Bordeaux for cucumber downy mildew are presented in table 2. These results differ from those for bean rust in several important respects. The critical toxicity values for bluestone and Bordeaux are similar, and both are much less than for bean rust, showing that copper is much more toxic to the cucumber downy-mildew organism than to the bean-rust fungus, and possibly that the downy-mildew sporangia are able to release copper from Bordeaux films on glass, while bean-rust urediospores are not, or have only weak copper-dissolving powers. At the LD 50 level of fungicidal action no marked differences are apparent between the results for cucumber downy mildew on glass and on leaves, while at the LD 95 level both bluestone and Bordeaux are much more effective per unit of copper on glass than on leaves. Because of the poor spreading properties of the sprays on leaves it is difficult to appraise these differences. Another important result shown in table 2, for which no explanation is offered, is the much greater increase in control resulting from adding spreader to the Bordeaux than in adding the spreader to the lime or bluestone.

TABLE 2.—*Comparative toxicity and protective value of lime, bluestone, and Bordeaux mixture for cucumber downy mildew*

| Fungicide | Germination on glass. Dosage per square decimeter for | | Protection on plants. Dosage per square decimeter for | |
|---|---|---|---|---|
| | 50 per cent reduction in germination | 95 per cent reduction in germination | 50 per cent reduction in number of lesions on leaves | 95 per cent reduction in number of lesions on leaves |
| CaO | 40 mg. CaO | 113 mg. CaO | 21 mg. CaO | 122 mg. CaO |
| CaO + 0.05 per cent spreader[a] | ..... | ..... | 5.2 mg. CaO | 33 mg. CaO |
| $CuSO_4 \cdot 5H_2O$ | 0.0056 mg. Cu | 0.049 mg. Cu | 0.008 mg. Cu | 2.2 mg. Cu |
| $CuSO_4 \cdot 5H_2O$ + 0.05 per cent spreader[a] | ..... | ..... | 0.003 mg. Cu | 0.45 mg. Cu |
| Bordeaux | 0.0074 mg. Cu | 0.060 mg. Cu | 0.012 mg. Cu | 1.7 mg. Cu |
| Bordeaux + 0.05 per cent spreader[a] | ..... | ..... | 0.0009 mg. Cu | 0.037 mg. Cu |

[a] 0.05 per cent phthalic glyceryl alkyd resin, used as a spreader in these tests on foliage, gave 45 per cent protection (average of 3 tests) when used alone.

## RELATION OF COPPER ABSORPTION BY LEAVES TO FUNGICIDAL PROTECTION

The injury from bluestone applied to leaves (Fig. 1) and the greater control of bean rust by Bordeaux than by bluestone (Fig. 2) suggested the absorption of copper by bean leaves in large amounts from bluestone but not from Bordeaux. To measure this copper absorption indirectly, and determine its effect on the protective value of copper, one set of bean leaves was

sprayed with 0.1 per cent bluestone, and opposite leaves of the same plants were sprayed with 0.1 per cent Bordeaux. These leaves were used for copper analysis or were inoculated with bean rust immediately or after being held for 4 hours in a moist chamber. The sprayed leaves on plants held in the moist chamber for 4 hours were still wet on removal. To determine copper the leaves were immersed in 0.5 per cent nitric acid and agitated occasionally for 20 minutes and then removed. It was hoped in this way to remove the copper from the surface of the leaves, but not the copper within the leaves. To this nitric acid extract 0.01 per cent sodium diethyl dithiocarbonate was added (1), and the copper was determined by comparison with standards of known copper content in a photoelectric colorimeter.

The results presented in table 3 indicate that over half the copper in bluestone spray was absorbed by the leaf in 4 hours if the leaf remained wet, while little if any of the copper in Bordeaux was so absorbed. Apparently the copper absorbed by the leaves had little if any function in protection

TABLE 3.—*Effect of incubating sprayed plants in a moist chamber on the surface deposit of copper, and on the protective value of sprays for bean rust. (Plants sprayed with 0.1 per cent bluestone, with or without lime as indicated, in all cases)*

| Copper determined or plants inoculated | Copper per square decimeter of leaves[a] | | Reduction in number of rust pustules on inoculated plants[b] | |
|---|---|---|---|---|
| | Sprayed with bluestone | Sprayed with Bordeaux | Sprayed with bluestone | Sprayed with Bordeaux |
| | *mg.* | *mg.* | *Per cent* | *Per cent* |
| Immediately after spraying | 0.56 | 0.50 | 79 | 100 |
| After 4 hours in a moist chamber | 0.24 | 0.50 | 56 | 99 |

[a] Average of 3 tests for each value.
[b] Average of 4 tests for each value.

from bean rust. Control was only slightly reduced by holding Bordeaux-sprayed plants in a moist chamber. It is apparent also that by holding bluestone-sprayed plants in a moist chamber disease control was reduced out of proportion to the loss of chemically determined surface copper. This is possible, because in addition to the absorption of surface copper by the leaves, nitrogenous materials, such as asparagin, may have diffused into the surface spray drops and reduced the protective value of the surface copper. In other tests, not reported here, the writer has found that the addition of asparagin to bluestone or Bordeaux decreased the protective value of these sprays for bean rust.

The above results further emphasize that an important function of lime in Bordeaux is to hold the copper in a form available and toxic to the rust fungus and relatively unavailable to the host.

### VARYING THE COPPER : LIME RATIO

The studies reported so far have been concerned with the toxicant applied as bluestone, as lime, or as Bordeaux made from equal amounts of

bluestone and lime. Since it has been shown that Bordeaux has considerably more protective value per unit of copper than bluestone, it might be expected that there would be a gradual increase in protective value of bluestone with increasing amounts of lime. Average results of 2 tests to determine the effect of the copper : lime ratio on the protective value of Bordeaux for bean rust are presented in figure 3. With dosages of 0.001 and 0.003 per cent bluestone, disease control was slight, results were rather erratic, and no effect of lime at dosages from 0.001 per cent to 0.1 per cent was apparent. With 0.01 per cent, 0.03 per cent, and 0.1 per cent bluestone, however, control was marked with bluestone alone, and the amount of control increased as the lime was increased from none to 0.1 per cent lime. These tests were not sufficiently refined to determine accurately the optimum copper : lime ratio for best disease control under these conditions but indicate that it was of the order of about 1 : 1.

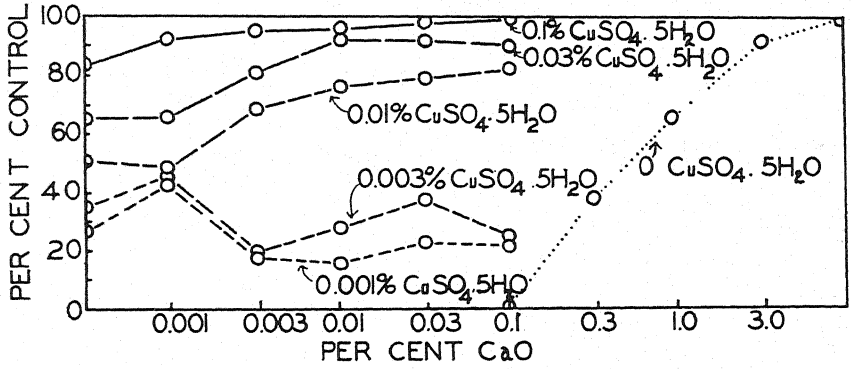



FIG. 3. Effect of varying the bluestone : lime ratio on the per cent control (= per cent reduction in number of rust pustules) of bean rust.

The effect of varying the bluestone : lime ratio on the eradicant value of Bordeaux was determined for bean powdery mildew. Average results of 2 tests, presented in figure 4, show that lime alone had very little eradicant action until a dosage of 0.1 per cent was reached; beyond this, powdery-mildew control was proportional to the dosage. Bluestone alone at 0.003 per cent caused about 38 per cent eradication; at 0.01 per cent, about 86 per cent; at 0.03 per cent, about 96 per cent; at 0.1 per cent, about 98 per cent; and at 0.3 per cent, 100 per cent. With all dosages of bluestone from 0.01 to 0.3 per cent the addition of increasing amounts of lime up to 0.3 or 1 per cent lime, caused a progressive reduction in the eradicant value of the spray. After 0.3 or 1.0 per cent lime was reached, an increase in lime caused an increase in control, but this increase apparently was due to the lime rather than the copper. Preliminary results, not presented here, show that bluestone applied as an eradicant spray for bean rust, a few hours after stomatal penetration had been effected, was more effective per unit copper than was Bordeaux. The depressing effect of lime on the eradicant value of blue-

stone for bean powdery mildew and bean rust is, therefore, apparently the opposite of its effect on the protective value of bluestone for bean rust.

## DISCUSSION

From published observations, snapdragon rust (*Puccinia antirrhini*) is apparently similar to bean rust with respect to the role of lime and host leaves in the action of Bordeaux mixture as a protective fungicide. According to Doran (3) bluestone was about 100 times (magnitude not clear from Doran's data) as toxic per unit of copper to the urediospores on glass as was Bordeaux mixture. Doran used Bordeaux mixture without spreaders and found it was ineffective in controlling snapdragon rust, but Green (5) and Foster (4) added spreaders to Bordeaux and secured fair to excellent control of the disease.

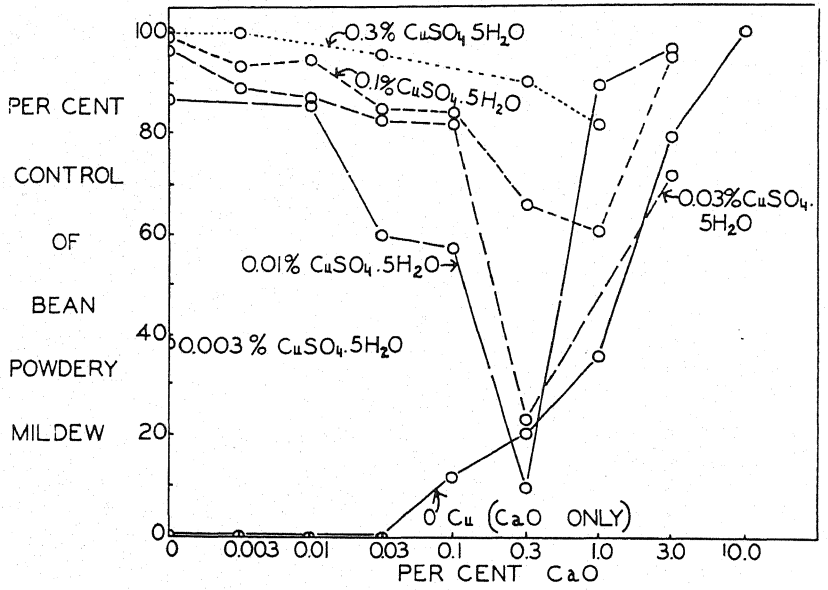



FIG. 4. Effect of varying the bluestone: lime ratio on the eradicant action of Bordeaux for bean powdery mildew.

With some fungi (Clark (2), and cucumber downy mildew in this paper) it would appear that Bordeaux mixture, on glass, and bluestone at the same copper dosage are about equally toxic. In the effect of the host in causing Bordeaux to be more active on leaves than on glass, as observed for bean rust, the writer knows of no comparable data in the literature.

Results reported here may have an important bearing on the application of *in vitro* toxicity to the evaluation of fungicides for the control of plant diseases. If many fungi behave as does bean rust, in that the same fungicide may be several hundred times more fungicidal on host surfaces than on glass, this would constitute a serious weakness to the *in vitro* testing of fungicides.

SUMMARY

Sprays consisting of lime alone, bluestone alone, and mixture of lime and bluestone (= Bordeaux mixture) were compared for their effect in inhibiting the germination of bean-rust urediospores and cucumber downy mildew sporangia, for protection from infection with bean rust and cucumber downy mildew, and for eradicating established infections of bean powdery mildew, at a range of dosages from below 50 per cent control to above 95 per cent control.

To give 95 per cent inhibition of germination of bean rust urediospores on glass required 300 mg. per sq. dm. of CaO, 0.60 mg. Cu as bluestone, or 64 mg. Cu as Bordeaux. To give 95 per cent reduction in number of rust pustules on plants required 124 mg. CaO, 2.6 mg. Cu as bluestone, or 0.18 mg. Cu as Bordeaux. For equivalent effectiveness it, therefore, required about 100 times as much copper in the form of Bordeaux as in the form of bluestone when the tests were on glass slides, but 10 times as much bluestone as Bordeaux when the tests were on leaves. To obtain equivalent effectiveness with Bordeaux, 350 times as much spray was required when the tests were on slides as when on leaves.

In similar tests with cucumber downy mildew, much less copper was required to inhibit germination than for bean rust, about the same amount of copper as bluestone or Bordeaux was required to give equivalent inhibition of germination on glass, and about the same amount of copper as bluestone or Bordeaux to give equivalent protection on plants, but much more copper was required to give 95 per cent protection than to give 95 per cent inhibition of germination. To give 95 per cent protection on leaves the addition of 0.05 per cent phthalic glyceryl alkyd resin as a spreader to the spray decreased the necessary amount of conventional fungicide to 27 per cent in case of lime, to 20 per cent in the case of bluestone, and to 2 per cent in case of Bordeaux.

Bean leaves sprayed with 0.1 per cent bluestone and held in a moist chamber for 4 hours showed by chemical determination only 43 per cent of the applied copper remaining on the surface of the leaves, and the control of bean rust was greatly reduced by this moist-chamber treatment, while there was no marked loss of copper or of rust control by holding Bordeaux-sprayed plants in a moist chamber under similar conditions. The results confirm other observations that leaves may absorb large quantities of copper from bluestone but not from Bordeaux.

When increasing amounts of lime were added to bluestone solutions the protective value of these mixtures for bean rust was progressively increased but the eradicant value for bean powdery mildew and bean rust was progressively decreased.

It is concluded that an important function of lime in Bordeaux mixture is to hold the copper in a form relatively unavailable and non-toxic to the host, but toxic to parasitic fungi, and an important function of the host may be to increase the effectiveness of the copper in some cases.

The relation of these results to the evaluation of fungicides is briefly discussed.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

LITERATURE CITED

1. CALLAN, THOMAS, and J. A. RUSSELL HENDERSON. A new reagent for the colorimetric determination of minute amounts of copper. Analyst 54: 650–653. 1929.
2. CLARK, JUDSON F. On the toxic properties of some copper compounds with special reference to Bordeaux mixture. Bot. Gaz. 33: 26–48. 1902.
3. DORAN, WILLIAM L. Rust of Antirrhinum. Massachusetts Agr. Exp. Stat. Bull. 202: 39–66. 1921.
4. FOSTER, W. R. Control of snapdragon rust *Puccinia antirrhini* D. and H. Sci. Agr. 18: 524–526. 1938.
5. GREEN, D. E. Antirrhinum rust II. The result of spraying. Jour. Roy. Hort. Soc. 61: 64–76. 1936.
6. HORSFALL, JAMES G., and R. F. SUIT. The lime factor in Bordeaux injury. (Abstract) Phytopath. 28: 9. 1938.
7. HOWARD, FRANK L. The value of testing fungicides in the laboratory before use in the field. Proc. Amer. Soc. Hort. Sci. 37: 409–414. 1939.
8. MARSH, R. W. Notes on a technic for the laboratory evaluation of protective fungicides. Trans. British Mycol. Soc. 20: 304–309. 1936.
9. MILLARDET, PIERRE MARIE ALEXIS. The discovery of Bordeaux mixture. Translation from the French by Felix John Schneiderhan. Phytopathological Classic 3: 1–25. 1933.
10. PRÉVOST, BÉNÉDICT. Memoir on the immediate cause of bunt or smut of wheat, and several other diseases of plants, and on preventives of bunt. Translation from the French by George Wannamaker Keitt. Phytopathological Classic 6: 1–95. 1939.
11. WILCOXON, FRANK, and S. E. A. MCCALLAN. Theoretical principles underlying laboratory toxicity tests of fungicides. Contrib. Boyce Thompson Inst. 10: 329–338. 1939.
12. YARWOOD, C. E. Onion downy mildew. Hilgardia 14: 595–691. 1943.

# TYPHULA SNOWMOLD OF PASTURE GRASSES[1]

C. C. WERNHAM[2] AND ST. JOHN P. CHILTON[3]

(Accepted for publication June 1, 1943)

In the spring of 1939 specimens of winter-killed Orchard grass, *Dactylis glomerata* L., collected at Storrs, Connecticut, by the junior writer, were examined. Fungus sclerotia embedded in the leaves and leaf sheaths indicated that low temperature alone may not have been responsible for all the damage. Low temperature, isolation plantings were made with these sclerotia. Two sclerotia-producing basidiomycetes were isolated, one very similar to *Typhula itoana* Imai, the other distinctly different. Cultures of these and other sclerotial fungi from turf grasses (5) were sent to Miss Remsberg (2) for identification. In her letter of April 4, 1940, Miss Remsberg wrote: "I am still working with the *Typhula* cultures you sent me. . . . It looks now as though #302 and #304 are *T. itoana*, #327 is a new species, . . . #303 looks familiar, but I'm not sure just which one it is yet." In addition to these cultures, authentic isolates of *Typhula* species were received from Miss Remsberg for comparative studies.

## LIST OF CULTURES

| *Culture number* | *Identification and record* |
|---|---|
| 302 | *Typhula itoana*—Akaroa Orchard grass. Storrs, Conn. Col. Chilton Apr. 1939; Isol. Wernham; Det. Remsberg. |
| 303 | *Typhula* sp.—Danish Orchard grass. Storrs, Conn. Col. Chilton Apr. 1939; Isol. Wernham; Det. Remsberg. |
| 304 | *T. itoana*—Danish Orchard grass. Storrs, Conn. Col. Chilton Apr. 1939; Isol. Wernham; Det. Remsberg. |
| 308 | *T. itoana*—Barley and wheat. Königsberg, Germany. Col. A. Volk; Det. Remsberg; Cornell Herb. 28090; Remsberg 93. |
| 309 | *T. itoana*—Barley and wheat. Sandpoint, Idaho. Col. Remsberg; Det. Remsberg; C. H. 27099; Remsberg 289. |
| 310 | *T. itoana*—Barley. Iwate Prefecture. Japan. Col. H. Tasugi; Det. Remsberg; C. H. 27095; Remsberg 283. |
| 311 | *T. itoana—Agrostis* sp. Ithaca, N. Y. Col. Chilton Apr. 1939; Isol. Wernham; Det. Wernham. |
| 316 | *T. itoana—Agrostis tenuis* Sibth. State College, Pa. Col. Isol. and Det. Wernham. |

[1] Authorized for publication on March 1, 1943, as paper No. 1168 in the Journal Series of the Pennsylvania Agricultural Experiment Station and as contribution No. 53 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the Northeastern States.

[2] Assistant Professor of Botany, The Pennsylvania State College.

[3] Formerly Agent of U. S. Regional Pasture Research Laboratory, now with the Department of Botany, Bacteriology and Plant Pathology, Louisiana State University, Baton Rouge, Louisiana.

324 *T. idahoensis*—*Agropyron cristatum* (L) Gaertn. Tetoma, Idaho. Col. C. W. Hungerford; Det. Remsberg; C. H. 27223; Remsberg 27.

325 *T. idahoensis*—Wheat. Bozeman, Mont. Col. P. A. Young; Det. Remsberg; C. H. 25153; Remsberg 291.

327 *Typhula* sp. *Agrostis* sp.—Wayzata, Minn. Col. L. J. Feser; Isol. Wernham; Det. Remsberg.

Unfortunately, Miss Remsberg never completed her identification of 303 and 327. At least they are not listed in her taxonomic treatment (2), nor in her pathological resume (3).

An investigation of *Typhula* snowmold of pasture and fine turf grasses was undertaken to ascertain: (a) A standardized method of inoculation under controlled conditions, (b) the host range of various isolates of *Typhula,* (c) the prevalence of physiogenic races, and, (d) the presence or absence of disease-resistant plants.

A preliminary experiment was devised to develop an inoculum and to determine the length of time necessary to obtain readily readable results from artificial inoculation.

## EXPERIMENTAL RESULTS

Inoculum was prepared as follows: to 500 g. of wheat or rye, in wide-mouth two-liter flasks, was added 4 g. $CaCO_3$ and 500 cc. water. The flasks were plugged with cotton and autoclaved at 17 lb. pressure for 30 to 60 min. After cooling, each flask of medium was inoculated with sclerotia from potato-dextrose agar cultures kept at 13° C. The time of incubation at this temperature varied from 6 to 8 weeks, depending on the isolate.

Meanwhile seed of barley, oats, rye, orchard grass, timothy, Kentucky bluegrass, and Astoria bent was planted in pots of sterilized soil in a greenhouse. When the plants were well past the seedling stage they were inoculated.

Each flask of inoculum was emptied on clean paper and thoroughly mixed, then roughly divided into aliquot parts. A part of the sclerotia-infested grain was scattered uniformly over the plants in each pot and gently rubbed into the soil. Sterile sand was scattered over the plants and washed down until the inoculum was completely buried. Uninoculated, sand-covered checks were used with each culture. Pots were placed in a low-temperature chamber, maintained at 2 to 8° C., and watered regularly to simulate the moisture conditions under melting snow. Once a month they were examined for evidence of sclerotia in the culms and leaves. At the end of 4 months, when sclerotia began to appear, the plants were taken up and the amount of infection determined. Evidence of infection was considered to be "presence of sclerotia imbedded in culms and leaves" (Table 1).

There was considerable variation among strains and species of *Typhula* tested. This variation may have been due to (a) strain or species differences, (b) time elapse of incubation period, (c) stage of maturity of plants,

TABLE 1.—*Results from preliminary inoculation tests with Typhula sp. based on presence of sclerotia embedded in culms and leaves. Plants inoculated July 19. Data taken December 10, 1939*

| Isolate | Number of seedlings diseased (D) or healthy (H) of | | | | | | | | | | | | | | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Barley | | Oats | | Rye | | Wheat | | Orchard grass | | Timothy | | Kentucky blue | | Astoria bent | | | |
| | D | H | D | H | D | H | D | H | D | H | D | H | D | H | D | H | D | H |
| *Typhula itoana* | | | | | | | | | | | | | | | | | | |
| 302 | 0 | 10 | 0 | 62 | 0 | 31 | 0 | 34 | 0 | 40 | 0 | 31 | 0 | 64 | 0 | 37 | 0 | 309 |
| 304 | 0 | 59 | 0 | 67 | 0 | 36 | 0 | 36 | 0 | 68 | 0 | 63 | 0 | 38 | 0 | 70 | 0 | 437 |
| 308 | 7 | 6 | 20 | 37 | 10 | 30 | 12 | 44 | 5 | 38 | 0 | 60 | 8 | 50 | 14 | 30 | 76 | 295 |
| 309 | 22 | 32 | 16 | 29 | 24 | 24 | 36 | 17 | 16 | 41 | 24 | 51 | 11 | 35 | 1 | 53 | 150 | 282 |
| 310 | 36 | 19 | 53 | 13 | 39 | 2 | 26 | 35 | 35 | 33 | 28 | 18 | 24 | 15 | 21 | 14 | 262 | 149 |
| 311 | ..... | ..... | 6 | 15 | 11 | 27 | 41 | 24 | 6 | 46 | 14 | 37 | 12 | 38 | 34 | 62 | 124 | 249 |
| 316 | 10 | 35 | 13 | 47 | 4 | 37 | 5 | 30 | 1? | 54 | 14 | 32 | 0 | 31 | 13 | 59 | 59 | 325 |
| *Typhula idahoensis* | | | | | | | | | | | | | | | | | | |
| 324 | 0 | 45 | 0 | 40 | 0 | 26 | 0 | 66 | 0 | 43 | 0 | 46 | 0 | 30 | 0 | 48 | 0 | 344 |
| 325 | 0 | 52 | 0 | 63 | 0 | 48 | 2 | 52 | 0 | 35 | 0 | 45 | 0 | 37 | 0 | 85 | 2 | 417 |
| Total | 75 | 258 | 108 | 373 | 88 | 261 | 122 | 338 | 62 | 398 | 80 | 383 | 55 | 338 | 83 | 458 | ..... | ..... |
| Checks | 0 | ..... | 0 | ..... | 0 | ..... | 0 | ..... | 0 | ..... | 0 | ..... | 0 | ..... | 0 | ..... | ..... | ..... |

(d) variety of plant used, (e) the method of reading data, or (f) to experimental error. If the complete stock of isolates were to be tested more variation could be expected.

The method of inoculation and environment for incubation were least subject to change. Since, from our limited surveys, the injury occurring in pastures appeared to be largely due to *Typhula itoana, T. idahoensis* could be excluded in subsequent trials.

In the first of these trials 10 young plants from seed of each host species or variety were grown in rows in flats. Triplicate plantings were made for each culture of *Typhula* to be tested. The small-seed species were first germinated in sand and later transplanted to the flats. Randomized plantings were introduced to avoid position effect in the flat. Inoculations were made as indicated above and the flats were distributed at random in the cold chamber. Subsequent care was limited to watering the flats at the intervals necessary to keep the sand moist.

At the conclusion of the low-temperature exposure period the flats were removed to the greenhouse and allowed to dry out sufficiently to permit easy removal of sand and excess inoculum by jarring and shaking the flats. Each row of plants was removed and the individual plants were separated. When it was expedient to do so, the excess sand and soil particles were washed off by gentle manipulation in a bucket of water.

It became evident that in some cases particles of clinging soil and débris could be easily mistaken for sclerotia, even under a hand lens. In such doubtful cases of infection, resort was made to water immersion. The plant under consideration was immersed in water contained in the top or bottom of a glass moist chamber under which a sheet of white paper was placed. The ease of this method in distinguishing sclerotia from clay and small pebbles is worthy of mention. Most of the cultures could be disposed of at the rate of one flat of 110 plants, an hour; but a few cultures regularly consumed 4–6 hours per flat (Table 2).

The criterion "presence of imbedded sclerotia in culms and leaves is evidence of infection" failed to give the true picture under the conditions of this experiment. Flats inoculated with cultures 302 and 304 were tedious to read. Sclerotia in the tissues were hard to find; when they were located it was often apparent that they occurred in portions of leaves buried under the sand. Mere saprophytism could be responsible for their presence. The abundance of sclerotia produced by all other isolates on all parts of the plants, even on the roots of the cereals (1, 4), seemed to indicate a positive parasitism. Remsberg (2), p. 71, cites *Dactylis glomerata* as a host of *Typhula itoana*. Except for cultures 302 and 304, our data confirm her findings. It is possible that these two strains were saprophytic. If this were true, then culture 303 may have been the true orchard-grass pathogen at Storrs, Connecticut, or the winter killing was purely physiogenic. In this experiment the cereals were invariably killed and often disintegrated to the point of being irrecoverable. The grasses in most cases were injured externally, but the crowns appeared to be viable.

TABLE 2.—*Results of inoculation tests of Typhula species on various grains and grasses, from three randomized plantings of 10 plants each, expressed as number of infected plants of total plants recovered. Inoculated May 23, 1940. Data taken October 8, 1940*

| Hosts | Planting date | Number of seedlings infected (I) and total (T) with indicated isolate number | | | | | | | | | | | | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 302[a] | | 304[a] | | 308 | | 309 | | 310 | | 316[b] | | 327[b] | | | |
| | | I | T | I | T | I | T | I | T | I | T | I | T | I | T | I | T |
| Bent, Astoria | 2–28–40 | 29 | 29 | 22 | 30 | 27 | 27 | 27 | 30 | 26 | 26 | 30 | 30 | 29 | 29 | 190 | 201 |
| Bent, Colonial | 2–28–40 | 27 | 30 | 28 | 30 | 30 | 30 | 28 | 29 | 28 | 28 | 29 | 29 | 21 | 28 | 191 | 204 |
| Barley | 4–26–40 | 14 | 27 | 11 | 28 | 23 | 28 | 23 | 28 | 29 | 29 | 22 | 26 | 12 | 30 | 134 | 196 |
| Kentucky bluegrass | 3– 8–40 | 24 | 29 | 23 | 27 | 29 | 29 | 27 | 27 | 30 | 30 | 22 | 25 | 14 | 25 | 169 | 192 |
| Oats | 4–26–40 | 20 | 26 | 13 | 29 | 29 | 29 | 27 | 29 | 27 | 28 | 24 | 27 | 17 | 29 | 157 | 197 |
| Orchard grass | 2–28–40 | 24 | 30 | 22 | 28 | 29 | 29 | 29 | 29 | 29 | 29 | 30 | 30 | 21 | 28 | 184 | 203 |
| Orchard grass, Danish 22385 | 3–11–40 | 22 | 25 | 16 | 26 | 19 | 19 | 24 | 25 | 26 | 26 | 28 | 29 | 26 | 29 | 161 | 179 |
| Orchard grass, Akaroa 118759 | 2–28–40 | 23 | 26 | 25 | 30 | 30 | 30 | 26 | 26 | 26 | 26 | 29 | 29 | 16 | 26 | 175 | 193 |
| Rye | 4–26–40 | 16 | 22 | 13 | 25 | 30 | 30 | 21 | 25 | 27 | 27 | 18 | 19 | 11 | 21 | 136 | 169 |
| Timothy | 2–28–40 | 23 | 27 | 16 | 27 | 25 | 29 | 23 | 29 | 27 | 27 | 27 | 27 | 14 | 29 | 155 | 195 |
| Wheat | 4–26–40 | 21 | 30 | 16 | 28 | 28 | 29 | 24 | 30 | 30 | 30 | 23 | 27 | 10 | 30 | 152 | 204 |
| Total | ..... | 243 | 301 | 205 | 308 | 299 | 309 | 279 | 307 | 305 | 332 | 282 | 298 | 191 | 304 | ..... | ..... |

[a] Sclerotia very scarce and hard to find; if present chiefly on buried leaves.
[b] Specimens of each infected host deposited in botany herbarium.

On the basis of the above results a third experiment was planned. The following features were incorporated: (1) Inclusion of a noninoculated check for the estimation of plant viability as affected by the treatment, (2) trimming-off of all excess leaf material previous to inoculation, (3) application of sand in such a manner that a minimum of plant tissue was buried, (4) more care taken to insure a perfect stand, and (5) incorporation of culture 303 into the test.

Unfortunately, such a careful set-up ended in near disaster, due to the fact that peat was used to conserve moisture in the flats and damping-off resulted. Much transplanting had to be done and a very uneven stand was obtained. In addition the inoculum became contaminated from standing too long. Because of the time consumed in preparation, it was decided, however, to salvage what remained and make the tests (Table 3). Certain

TABLE 3.—*Results of inoculation tests of Typhula species on various grains and grasses, from three randomized plantings expressed as number of infected plants of total plants recovered. Inoculated April 1, 1941. Data taken August 14, 1941*

| Host | Number of seedlings infected (I) and total (T) with indicated isolate No. | | | | | | Total | | Check | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 303 | | 310 | | 327 | | | | | |
| | I | T | I | T | I | T | I | T | I | T |
| Bent, Astoria | 30 | 30 | 24 | 29 | 31 | 31 | 85 | 90 | 0 | 30 |
| Bent, Colonial | 29 | 29 | 23 | 29 | 30 | 30 | 82 | 88 | 0 | 30 |
| Barley | 33 | 34 | 33 | 34 | 32 | 32 | 98 | 100 | 0 | 31 |
| Ky. bluegrass | 30 | 30 | 28 | 30 | 28 | 30 | 86 | 90 | 0 | 29 |
| Oats | 27 | 29 | 33 | 34 | 29 | 30 | 89 | 93 | 0 | 30 |
| Orchard grass | 32 | 32 | 29 | 30 | 30 | 30 | 91 | 92 | 0 | 30 |
| Orchard grass, Danish 22385 | 30 | 30 | 30 | 30 | 30 | 30 | 90 | 90 | 0 | 30 |
| Orchard grass, Akaroa 118759 | 29 | 29 | 28 | 30 | 30 | 30 | 87 | 89 | 0 | 30 |
| Rye | 16 | 22 | 20 | 24 | 28 | 30 | 64 | 76 | 0 | 26 |
| Timothy | 30 | 30 | 26 | 30 | 30 | 30 | 86 | 90 | 0 | 30 |
| Wheat | 30 | 30 | 29 | 31 | 28 | 29 | 87 | 90 | 0 | 30 |
| Total | 316 | 325 | 303 | 331 | 326 | 332 | ..... | ..... | 0 | 326 |

positive results were obtained. The treatment had little effect on the viability of the uninoculated check plants. The clipped and trimmed plants were abundantly covered with sclerotia. Culture 303 was equal in pathogenicity to 310 and 327.

The meager results obtained from this experiment left the peculiar results of the other two orchard grass isolates, 302 and 304, unexplained. This reaction was a general one applicable to all plants tested rather than a specific one limited to Orchard grass alone. Nor did it resemble the reactions of other cultures of *Typhula* on plants from the same species. Unfavorable temperature or time of exposure could have been responsible for the results. Of these factors, the latter offered the most promise for further tests. Varying the temperature involved too much space and equipment for the length of time required. For this reason it was decided to limit a final experiment

TABLE 4.—*Results of inoculation tests of Typhula species on various grasses, from two random plantings of 10 plants each expressed as number of infected plants of total plants recovered. Inoculated February 23, 1942. Data taken September 23, 1942*

| Hosts | Number of seedlings infected (I) and total (T) with indicated isolate numbers | | | | | | | | | | | | | | | | Total | | Check | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 302[a] | | 303[b] | | 304[a] | | 308 | | 309 | | 310 | | 316 | | 327 | | | | | |
| | I | T | I | T | I | T | I | T | I | T | I | T | I | T | I | T | I | T | I | T |
| Kentucky bluegrass | 17 | 20 | 18 | 18 | 15 | 20 | 20 | 20 | 19 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 149 | 158 | 0 | 19 |
| Orchard grass | 18 | 20 | 20 | 20 | 18 | 20 | 19 | 19 | 18 | 19 | 20 | 20 | 20 | 20 | 19 | 19 | 152 | 156 | 0 | 20 |
| Orchard grass, Danish 22385 | 19 | 19 | 19 | 19 | 20 | 20 | 19 | 19 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 157 | 157 | 0 | 20 |
| Orchard grass, Akaroa 118759 | 19 | 19 | 20 | 20 | 19 | 19 | 17 | 17 | 20 | 20 | 20 | 20 | 18 | 18 | 19 | 19 | 152 | 152 | 0 | 19 |
| Timothy | 12 | 20 | 18 | 18 | 12 | 14 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 19 | 19 | 141 | 151 | 0 | 20 |
| Total | 85 | 98 | 95 | 95 | 84 | 93 | 95 | 95 | 97 | 99 | 100 | 100 | 98 | 98 | 97 | 97 | ..... | ..... | 0 | 98 |

[a] Sclerotia small and relatively sparse, though more numerous than in previous trials.
[b] Specimens on all hosts deposited in botany herbarium—very pathogenic.

to the time variable, to include only grasses and to limit these to duplicate plantings (Table 4).

In spite of the lengthened exposure (7 months instead of 4.5 months), the check plants remained remarkably viable. Inoculated plants were nearly all killed. Cultures 303, 308, 309, 310 and 316 caused greatest injury. This was easily determined without disturbing many plants. Least destructive were cultures 302, 304 and 327. Of these 302 and 304 showed a slight increment in number of sclerotia present, but these were small compared to their size in culture.

## DISCUSSION

The particular value of the work herein reported lies in the fact that most pasture grasses have proved quite susceptible to the species of *Typhula* commonly found on grains and grasses (2). Under the conditions of our experiment there was very little evidence of disease resistance, although some noninfected plants usually were found. The small number of plants used in these tests hardly serve as a basis for determining this character. That disease resistance occurs in some species has been reported by Remsberg and Hungerford (4) and demonstrated by Wernham (5). The importance of disease resistance is evident in any grass-improvement program provided that the disease is economically serious in the area. The extent of winter injury in pastures is not very well known, probably because the pastures are not examined early enough in the season. It is impossible, therefore, to estimate the percentage of winter injury that is due to disease. It seems logical from the data presented that the amount and severity of winter injury due to snowmold would depend on the type and duration of certain weather phenomena. A dry, open winter would present less favorable conditions than a wet, snowy one of long duration. The senior writer has observed this to be the case in field-plot inoculations on fine turf.

Whatever importance snowmold of pasture grasses may assume, an experimental approach to the study of disease resistance has been established under conditions of artificial inoculation. Independent of the vagaries of the weather to which field-plot techniques are subjected, the method provides for positive results at any season on a scale of operation hitherto unattained (4). In addition it suggests that artificial covering may be substituted for snow in field-plot trials.

The evidence indicates, too, that not all the organisms responsible for winter killing or injury have been determined. Nor is it safe to assume that the presence of a familiar looking organism is evidence of its pathogenicity, regardless of its previously established record. There does not seem to be any evidence of pathogenetic specialization among the species of *Typhula* tested.

## SUMMARY

A method of testing grasses and cereals for their reaction to *Typhula* sp. under controlled conditions is described.

An undescribed species of *Typhula* is probably responsible for some winter killing of Akaroa and Danish orchard grass.

The more common pasture grasses of the Northeastern States are highly susceptible to *Typhula* snowmold under artificial conditions.

Most of the isolates of *Typhula* sp. tested herein are pathogenic to all the species of grasses included in the tests.

Pathogenetic races among the species of *Typhula* considered were not revealed under the conditions of our experiments.

A statement regarding the presence or absence of plants resistant to *Typhula* sp. is not warranted from our results.

## LITERATURE CITED

1. HUNGERFORD, C. W. A serious disease of wheat caused by *Sclerotium rhizoides* in Idaho. Phytopath. 13: 463–464. 1923.
2. REMSBERG, RUTH E. Studies in the genus *Typhula*. Mycologia 32: 52–96. 1940.
3. ———. The snowmolds of grains and grasses caused by *Typhula itoana* and *Typhula idahoensis*. Phytopath. 30: 178–180. 1940.
4. ———, and C. W. HUNGERFORD. Certain sclerotium diseases of grains and grasses. Phytopath. 23: 863–874. 1933.
5. WERNER, C. C. New facts about eastern snowmold. Phytopath. 31: 940–493. 1941.

# SOIL FUMIGATION WITH CHLOROPICRIN FOR CONTROL OF THE ROOT KNOT NEMATODE, HETERODERA MARIONI[1]

A. L. TAYLOR

(Accepted for publication May 7, 1943)

## INTRODUCTION

It is a well-recognized fact that the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, is one of the worst plant pests known. The possibility of control of this pest by means of volatile poisons, applied to the soil before planting a host crop susceptible to damage, has long been recognized, and numerous chemicals have been more or less thoroughly tested for this purpose. One of the most promising of these is chloropicrin.[2]

## REVIEW OF LITERATURE

Chloropicrin was first employed as a nematocide in 1919 (11), but little was published on the subject until 1932. In that year Johnson and Godfrey (10) reported that good control of root knot could be obtained by the use of 163 lb. of the chemical per acre. In their experiments it was applied by introducing small amounts into holes in the soil, 5 or 6 inches deep and 18 inches apart. In 1934, Godfrey, Oliveira, and Hoshino (8) reported that good control of root knot had been obtained in Hawaii by applying 250 to 400 pounds of chloropicrin per acre to loose soil without excessive moisture. Applications were spaced no more than 18 inches apart, and a gas-impervious cover of glue-coated paper was placed over the soil after treating. In 1935, Godfrey (6) reported that applications of 130 to 170 lb. of chloropicrin per acre in pineapple fields had given sufficiently good control of root knot to produce a substantial increase in yield. Neller and Allison (12) in 1935, designed a machine for the rapid application of chloropicrin and found that good control of root knot was obtained by the use of 203 and 405 pounds of chloropicrin per acre. Newton, Bosher, and Hastings (14) devised a method for determining spacing of injections of soil fumigants in 1937 and obtained fair control of root knot in greenhouse benches by the use of 1 ml. of chloropicrin per square foot.

In 1939, Howard, Stark, and Smith (9) reported that surface watering of the soil was as effective as gas-impervious covers in increasing the efficacy

[1] Cooperative investigations by the Division of Nematology, Bureau of Plant Industry, United States Department of Agriculture, and the Georgia Coastal Plain Experiment Station, Tifton, Georgia.

[2] Chloropicrin ($CCl_3NO_2$) is a colorless liquid with a specific gravity of 1.63. It vaporizes easily, and the vapor is extremely irritating to the eyes, causing them to water profusely. Larger concentrations taken into the lungs cause violent coughing, nausea, and vomiting. These effects are produced by concentrations far below the lethal point and are only temporary, usually being relieved after a few minutes in fresh air. The liquid is highly irritating to the skin, and care should be taken to protect the hands and face while handling it. If spilled on clothing, the garment affected should be removed at once. It is not inflammable or explosive. If the user transfers it from one vessel to another only in the open air and takes ordinary precautions, it is not particularly dangerous to handle. However, any person susceptible to or afflicted with a respiratory disease should use full-face gas-mask protection, even while working out of doors.

of chloropicrin fumigations. In the same year, Chitwood (1) further refined the method of determining soil spacing for fumigants, and Taylor (16) developed mathematical formulas for calculating optimum amounts for use. In 1940, Young (18) reported good control of root knot by the use of 300 to 600 lb. of chloropicrin per acre, and found that several sorts of glue were suitable for coating paper to use in covering the soil after treatment. In the same year Chitwood (2) reported that root knot had been controlled in greenhouses and in the field by placing doses of a mixture containing 1.5 ml. of chloropicrin and 8.5 ml. of ethylene dichloride[3] in the soil in holes 10.5 inches apart in rows 9 inches apart. Taylor and McBeth (17) reported in 1941 that root knot of watermelons could be greatly reduced in the field by means of "spot treatments," involving only the small portion of the soil occupied by the roots of the plants, with considerable economy of material. Also in 1941, Chitwood (3) reported that root knot and fungus diseases of gerberas in greenhouse soil, and root knot and fungus diseases of tomatoes in the field, could be controlled by the mixture of chloropicrin and ethylene dichloride mentioned above.

Chitwood and Newhall (4) reported in 1942 that fall applications of chloropicrin, at the rate of 2 ml. per hole, with the holes spaced 10.5 inches apart in the soil, or a mixture of 1 part chloropicrin and 9 parts ethylene dichloride applied at the rate of 10 ml. to the hole, with the same spacing, gave good control of onion bloat caused by the nematode *Ditylenchus dipsaci* (Kühn) Filipjev. In this year also, Newhall and Stark (13) reported excellent control of *Heterodera marioni* on a spring crop of tomatoes by treating the soil the previous fall with chloropicrin at the rate of 10.5 pounds per 1000 square feet (460 pounds per acre) or with a 1 : 9 mixture of chloropicrin and ethylene dichloride at the rate of 1,824 pounds per acre. Both were applied at 10-inch intervals in holes 4 to 5 inches deep, 2-ml. doses of chloropicrin or 10-ml. doses of the mixture being used. Chloropicrin has also been found to be highly effective against insects (15) and fungi (7) in the soil.

## EXPERIMENTS

The experiments reported herein were begun in the summer of 1936 for the purpose of testing results obtained by previous investigators and developing a dependable method of using chloropicrin against the root-knot nematode. No other plant-parasitic nematodes, insects, fungi, or bacteria were considered.

In all the work reported, the soil used was Norfolk sandy loam in a field near Tifton, Georgia, that had been used several years for root-knot-susceptible crops and had a moderate infestation of root-knot nematodes. All experiments were performed under outdoor conditions during the spring, summer, and fall.

[3] Ethylene dichloride ($C_2H_4Cl_2$) vapors are highly toxic, inflammable, and explosive. Persons handling this substance should be extremely careful of fires and should wear full-face gas-mask protection.

Basic procedure was the same in all cases. The soil was prepared by plowing and leveling, the chloropicrin injected about 6 inches beneath the soil surface by means of carefully calibrated applicators, especially designed for the purpose, and the remaining soil population determined by use of the indicator-plant method. This method was tested by Godfrey (5), who recommended that a quick-growing crop, highly susceptible to root knot be planted at the rate of one plant per square foot on the soil to be tested. The plants are allowed to grow long enough to be invaded by most of the nematodes in the soil but not long enough to allow reproduction and secondary infection to occur. This period is about 30 days when soil temperatures are above 20° C. The roots are then removed with ordinary care, examined, and classified as infected or not infected. The correlation between the percentage of infected plants and the nematode population of the soil is sufficiently high to enable fairly accurate estimates of the soil population to be made and certainly high enough to show the large changes in population that might be expected to follow successful control measures. The system used in the present study followed this method, except that indicator plants often were allowed to grow from 6 weeks to 2 months, permitting the development of secondary infection and increasing the chances of detecting very small populations.

Precautions were taken during the growing period to prevent contamination of the plots by nematodes brought in by running water and cultivating tools. Border effects due to migration of nematodes into the plots were eliminated by examining only plants from the central portions of the plots and rejecting those growing within 2 feet of the edge. Indicator plants were invariably examined with a dissecting microscope and were classified as infected if even one root-knot nematode was found. Fifty to 100 plants were examined from each plot.

Results of the experiments are given in the tables as percentages of infected indicator plants. Differences in averages are referred to as "significant" when statistical analysis of the results indicated that odds were at least 19 to 1 that results were not due to chance, and as "highly significant" when the odds were 99 to 1 that results were not due to chance.

The chloropicrin applied was the standard commercial grade (99 per cent pure). Applications always were spaced so that adjacent application points formed equilateral triangles, and the application distances given are the length of the sides of these triangles (16). Soil temperatures were always measured at a depth of one foot. Diurnal variation in soil temperature at this depth usually is only 1° to 2° C. in southern Georgia.

Experiment No. 1 consisted of 25 plots, each 16 by 16 feet, arranged in a Latin square. Rates of application of the chloropicrin were: None, 50, 100, 200, and 400 lb. per A. Application points were spaced 16 inches apart. The soil temperature at the time of treatment was 25° C. Soil moisture was not determined. Plots were covered with overlapping strips of mulch paper immediately after treating and uncovered 4 days later. Ex-

amination of the Whippoorwill cowpeas, *Vigna sinensis* (L.) Haussk., used as indicator plants, gave the results presented in table 1.

TABLE 1.—*Percentages of root-knot infected indicator plants in plots treated with chloropicrin and covered with mulch paper*

| Treatment (pounds chloropicrin applied per acre) | Infected plants in plots after application of chloropicrin | | | | | |
|---|---|---|---|---|---|---|
| | Row 1 | Row 2 | Row 3 | Row 4 | Row 5 | Average[a] |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 0 | 56 | 50 | 27 | 14 | 5 | 30.4 |
| 50 | 17 | 7 | 1 | 2 | 0 | 5.4 |
| 100 | 5 | 5 | 0 | 0 | 0 | 2.0 |
| 200 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 400 | 0 | 0 | 0 | 0 | 0 | 0.0 |

[a] Differences between control and all treatments significant; no significant difference between treatments.

Experiment No. 2 was a 25-plot Latin square treated with chloropicrin at the following rates: None, 50, 100, 150, and 200 lb. per A. Application points were spaced 16 in. apart. The soil temperature at time of treating was 20° C. Soil moisture content was not determined. No paper covers were used after treatment. Instead, the plots were sprinkled with enough water to wet the top inch or two of soil, forming a "water seal" to prevent too rapid escape of the fumes. Squash (*Cucurbita maxima* Duchêsne) was used as an indicator crop. Results are given in table 2.

TABLE 2.—*Percentages of root-knot infected indicator plants in plots treated with chloropicrin and water-sealed*

| Treatment (pounds chloropicrin applied per acre) | Infected plants in plots after application of chloropicrin | | | | | |
|---|---|---|---|---|---|---|
| | Row 1 | Row 2 | Row 3 | Row 4 | Row 5 | Average[a] |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 0 | 86 | 32 | 30 | 36 | 68 | 50.4 |
| 50 | 45 | 18 | 30 | 12 | 8 | 22.6 |
| 100 | 29 | 41 | 2 | 11 | 8 | 18.2 |
| 150 | 3 | 4 | 0 | 1 | 3 | 2.2 |
| 200 | 2 | 6 | 0 | 4 | 2 | 2.8 |

[a] Differences between control and all treatment averages highly significant; difference between 100-lb. and 150-lb. treatments significant.

Experiment No. 3 consisted of 40 randomized plots, each 16 by 16 feet. Chloropicrin was applied to 32 of these in 4 groups of 8 plots each at the rates of 50, 100, 150, and 200 lb. per A.; and the remaining 8 plots were left untreated. As the experiment was originally designed to explore the relation of soil temperature and moisture to chloropicrin fumigations, groups of plots were treated at various temperatures from 18° to 25° C. and at soil moistures ranging from 2 to 8 per cent. Spacing of applications was 16 inches. No cover or water seal was used. As there were no significant

differences in the temperature and moisture portions of the experiment, table 3 gives the percentages of infected Whippoorwill cowpea indicator plants grouped according only to amounts of chloropicrin used.

As the only variation in technique between this experiment and the two previously described was the omission of the cover or water seal, the results indicated that successful fumigation cannot be expected when neither of these is used.

In another experiment a maximum of 200 lb. of chloropicrin was applied to soil having a moisture content of only 2 per cent. Although glue-coated kraft paper covers, with the joints lapped 6 inches and glued and the edges buried 8 inches in the soil, were used, no appreciable effect on the root-knot nematode population of the soil was produced.

TABLE 3.—*Percentages of root-knot infected indicator plants from plots treated with chloropicrin without cover or water seal*

| Treatment (pounds chloropicrin applied per acre) | Infected plants in plots after application of chloropicrin | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Block 7 | Block 8 | Average[a] |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 0 | 36 | 57 | 49 | 26 | 12 | 31 | 37 | 33 | 35.1 |
| 50 | 24 | 62 | 5 | 27 | 57 | 42 | 65 | 49 | 41.3 |
| 100 | 49 | 1 | 38 | 63 | 67 | 45 | 31 | 26 | 40.0 |
| 150 | 1 | 33 | 82 | 60 | 52 | 42 | 31 | 25 | 40.7 |
| 200 | 31 | 6 | 42 | 0 | 8 | 37 | 58 | 33 | 26.8 |

[a] No significant differences.

That chloropicrin might have important effects on germination of seed under certain conditions was demonstrated by an experiment in 1940. A block of plots was treated with chloropicrin at rates of 200, 300, and 400 lb. per A., with 4 plots to each treatment and 4 nontreated control plots. Soil moisture at time of treating was only 6 per cent, but some rain fell on each of the 8 days after the chemical was applied. An indicator crop of cowpeas, planted 5 days after treating, failed to emerge on the plots receiving 400 lb. of chloropicrin per acre. Approximately 10 per cent of the seed on the 300 lb. per A. plots germinated, 26 per cent on the 200 lb. per A. plots and 80 per cent on the nontreated checks. A second indicator crop, planted 1 month after treating, grew normally. Apparently normal escape of the fumes from the soil had been prevented by the daily rains, and enough remained when the first indicator crop was planted to injure the seed.

The experience gained in the previous work made possible the formulation of a system of using chloropicrin, which was tested in 1940. The procedure in detail was: The plots were plowed, harrowed, and leveled, so that the soil was well broken up and very loose at the time of treating. Soil temperature was 21° C. and soil moisture was 6.7 per cent. Sixteen 10 by 10 foot plots were laid out, raked smooth, and lightly sprinkled with water.

The soil surface was marked off in a grid of rectangles, 12 by 14 inches in size. The applicator was set to place the chemical 6 inches beneath the soil surface. Working along the lines 12 inches apart, the measured applications were placed at the intersections of the marked lines on the odd-numbered rows and halfway between these intersections on the even-numbered rows. Holes made by the applicator spike were covered with soil by the operator immediately after the chemical was placed. When chloropicrin had been applied to the whole plot, it was again raked and sprinkled with enough water to wet the top inch or two of soil. This completed the treatment. The plots were left undisturbed for a week to allow escape of the chemical, and then planted.

The 16 plots were randomized according to the Latin square method, and 4 plots assigned to each of the following treatments:

(A) No chloropicrin.

(B) 1.1 ml. of chloropicrin per application point (150 lb. per A.).

(C) 1.4 ml. of chloropicrin per application point (200 lb. per A.).

(D) 1.8 ml. of chloropicrin per application point (250 lb. per A.).

Squash were used as indicator plants and examined 6 weeks after planting. Results of the examination are presented in table 4.

TABLE 4.—*Percentages of root-knot infected indicator plants from plots treated with chloropicrin in 1940. Applications spaced at 14-inch intervals and water seal used*

| Treatment (pounds chloropicrin applied per acre) | Infected plants in plots after application of chloropicrin | | | | |
|---|---|---|---|---|---|
| | Row 1 | Row 2 | Row 3 | Row 4 | Average[a] |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 0 | 95 | 93 | 79 | 74 | 85.2 |
| 150 | 10 | 7 | 17 | 19 | 13.2 |
| 200 | 3 | 0 | 0 | 0 | 0.7 |
| 250 | 0 | 2 | 0 | 0 | 0.5 |

[a] Differences between control and all treated plots highly significant; differences between 150- and 200-pound treatments significant.

In the experiments reported above, the chloropicrin was applied when the soil contained no undecayed roots, as it was presumed that these would protect the contained nematodes from the effects of the fumigation. In commercial greenhouses, the time required for the decay of the roots of one crop, before fumigating the soil and planting another is often very important. An experiment designed to test the effect of undecayed roots in the soil on the efficiency of chloropicrin fumigation was performed in 1942. An infested plot, 50 by 50 feet, was planted to tomatoes (*Lycopersicon esculentum* Mill.), which were allowed to grow for 6 weeks. At the end of this period, the main roots of the plants were heavily infected with root knot, with many large galls 1 or 2 cm. in diameter. The plot was then plowed, covering the tomato plants completely. On the same day, the plot was sub-

divided to form 25 plots, each 10 by 10 feet. These were randomized by the Latin square method, with 5 plots in each of 5 treatments. The first group of plots (A) was the nontreated control. Chloropicrin at the rates of 200 and 300 lb. per A. was applied, on the same day the plots were plowed, to the second group of plots (B) and the third group of plots (C), respectively. Twenty days later, when the tomato roots had entirely disintegrated, the fourth (D) and fifth (E) groups of plots were treated with 200 and 300 lb. of chloropicrin per A., respectively. Thus the experiment consisted of control plots, plots treated when the roots were fresh, and those treated when the roots had entirely decayed, with chloropicrin applied to each of the latter two groups at 200 and at 300 lb. per A. Treatment procedure was as described for the preceding experiment. An indicator crop of cantaloupes (*Cucumis melo* L.) was planted and examined after growing for 1 month. Results of the examination are given in table 5.

TABLE 5.—*Percentages of root-knot infected indicator plants (cantaloupes) from plots treated with chloropicrin and containing infected tomato roots*

| Treatment (pounds chloropicrin applied per acre) and condition of tomato roots | Infected plants in plots after application of chloropicrin | | | | | |
|---|---|---|---|---|---|---|
| | Row 1 | Row 2 | Row 3 | Row 4 | Row 5 | Average[a] |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| (A) None | 86 | 96 | 100 | 96 | 98 | 95.2 |
| (B) 200 (Roots fresh) | 0 | 27 | 78 | 54 | 26 | 37.0 |
| (C) 300 (Roots fresh) | 0 | 20 | 28 | 2 | 8 | 11.6 |
| (D) 200 (Roots decayed) | 2 | 6 | 12 | 0 | 0 | 4.0 |
| (E) 300 (Roots decayed) | 0 | 0 | 6 | 0 | 0 | 1.2 |

[a] Difference between control plots (A) and all treated plots highly significant; difference between B and C significant; differences between C, D, and E not significant.

## DISCUSSION AND CONCLUSIONS

From the practical standpoint, satisfactory control of the root-knot nematode might be defined as an effected reduction in the soil population of this nematode to the point where at least 90 per cent of the indicator plants escape infection in the early stages of growth. Under these circumstances, about a year would be required for the number of nematodes again to reach the point where reductions in crop yields would be noticeable, even if the soil were used continuously for highly susceptible crops.

The results of the experiments indicate that this degree of control can be obtained under Tifton, Georgia, conditions by the use of a minimum of 200 pounds of chloropicrin per acre. Insignificantly better results were obtained with larger amounts. Smaller applications did not always give satisfactory control.

The condition of the soil at the time of application and the use of some device to confine the fumes to the soil are as important as the amount of chloropicrin used. Fumigation is effective within the entire soil-temperature range tested, 20° to 27°; furthermore, data from experiments not reported here indicate that successful fumigations can be made at temperatures even as low as 15° C. Fumigations made when the soil was moderately damp (moisture content about 6 per cent) were good, but those made when the soil was very dry (moisture content 2 per cent) were not successful, even when covers were used. While no data are available, it is probable that excessive soil moisture would prevent successful soil fumigation, since the diffusion of the fumes through the soil interspaces would be hindered by the water.

Fumigations made without the use of either paper covers or water seals were not successful. Mulch paper, and wetting the top inch or two of soil with water were effective in confining the fumes.

Results of the experiment with undecayed roots indicate that these interfere seriously with the efficiency of the fumigation. Where 200 pounds of chloropicrin per acre were used, satisfactory control was not obtained when fresh roots were in the soil, although the reduction in population produced by this amount after the roots had disintegrated was very satisfactory. When the amount of chloropicrin was increased to 300 pounds, a fair degree of control was obtained, even with undecayed roots in the soil. However, control in this case would be considered a little less than satisfactory, since two of the plots had 20 per cent or more of infected indicator plants.

In the sandy loam soils used in these experiments, applications can be spaced at 14- to 16-inch intervals. It is safe to assume that chloropicrin would be effective wherever the soil is open enough to permit ready diffusion of the fumes, but it is probable that optimum spacing and amounts would vary greatly. For this reason, general recommendations cannot be made. Prospective users are advised to experiment on a small scale before attempting large and extensive applications.

In one experiment, seeds planted before all of the chloropicrin had diffused out of the soil were injured, but this case was exceptional. No injury was observed to seeds planted one week after treating in the 1940 experiment.

Fumigation of one acre of soil with 200 pounds of chloropicrin would cost $160 for the chemical alone at minimum present prices. Application requires about 1 man-hour per 1,000 square feet of soil when a hand applicator of efficient design is used. It is apparent that the use of chloropicrin is economically practical only where crops of high value are to be grown. In greenhouses and seedbeds, it can replace steam sterilization in some cases or can be used where steam is not available. Other uses might be found in vegetable gardens, in seed beds, or in ornamental flower beds. In the field, only crops of high unit value would repay the investment. Here considerable savings may be made by the use of the spot-treatment technique (17). In this system, only the portion of the soil to be occupied by the roots of the

plants is fumigated. Cost of the chemical for watermelons in hills spaced 8 feet apart is about $5 per acre. Cost for other crops would vary according to the proportion of the soil occupied by the plant roots. Apparently it is impractical to eliminate the nematodes entirely, so the treatment would have to be repeated periodically, possibly every year where the soil is used continuously for susceptible crops.

## SUMMARY

The nematocidal effect of chloropicrin was tested in the field against the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. Satisfactory control was obtained by the use of 200 pounds of the chemical per acre in sandy loam soil containing no undecayed roots. Applications were made by placing measured quantities of chloropicrin in holes in the soil 6 inches deep and spaced at 14- to 16-inch intervals. Immediately after treating, the soil was sprinkled with enough water to wet the top inch or two, or was covered with mulch paper or glue-coated kraft paper. Applications made when the soil-moisture content was only 2 per cent or when no cover or water seal was used failed to produce satisfactory control. Amounts of chloropicrin less than 200 pounds per acre did not always produce satisfactory results.

When the soil contained fresh infected tomato roots, poor control was obtained with 200 pounds of chloropicrin per acre and fair control with 300 pounds per acre. In the same experiment, 200 pounds per acre applied after the infected roots had disintegrated, produced good control.

Germination of seed was partly prevented in one experiment where the indicator crop was planted before the chloropicrin had fully escaped from the soil.

## LITERATURE CITED

1. CHITWOOD, B. G. A rapid method of determining k values of nematocides. Proc. Helminthol. Soc. (Washington) 6: 66–70. 1939.
2. ————. Ethylene chloride for soil sterilization against nematodes. Flor. Exch. 95 (11): 9. 1940.
3. ————. Soil treatments with volatile liquids for control of nematodes. Phytopath. 31: 818–824. 1941.
4. ————, and A. G. NEWHALL. The efficacy of certain nematocides in the control of onion bloat in muck soil. Phytopath. 32: 254–258. 1942.
5. GODFREY, G. H. Indicator plants for measuring soil populations of the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. Soil Sci. 38: 3–27. 1934.
6. ————. Experiments on the control of the root-knot nematode in the field with chloropicrin and other chemicals. Phytopath. 25: 67–90. 1935.
7. ————. Control of soil fungi by soil fumigation with chloropicrin. Phytopath. 26: 246–256. 1936.
8. ————, JULIETTE OLIVEIRA, and HELENE M. HOSHINO. Increased efficiency of chloropicrin for nematode control with better confinement of the gas. Phytopath. 24: 1332–1340. 1934.
9. HOWARD, F. L., F. L. STARK, and J. B. SMITH. Chemical control of nematodes in tomato greenhouses. Phytopath. 29: 11. 1939.
10. JOHNSON, M. O., and G. H. GODFREY. Chloropicrin for nematode control. Indus. and Eng. Chem. 24: 311–313. 1932.
11. MATTHEWS, D. J. Report of the work of the W. B. Randall research assistant. Cheshunt Exp. and Res. Stat. Ann. Rpt. 5: 18–21. 1919.
12. NELLER, J. R., and R. V. ALLISON. A machine for the subsurface treatment of soils with chloropicrin and with carbon bisulphide for nematode control under field conditions. Soil Sci. 40: 173–174. 1935.

13. NEWHALL, A. G., and F. L. STARK, JR. Chloropicrin and ethylene dichloride for root-knot nematode control. Phytopath. 32: 626–630. 1942.
14. NEWTON, WILLIAM, J. E. BOSHER, and R. J. HASTINGS. The treatment of glasshouse soils with chloropicrin for control of *Heterodera marioni* (Cornu) Goodey, and other soil pathogens. Canad. Jour. Res. C. (Bot. Sci.) 15: 182–186. 1937.
15. STONE, M. W., and ROY E. CAMPBELL. Chloropicrin as a soil insecticide for wireworms. Jour. Econ. Ent. 26: 237–240. 1933.
16. TAYLOR, A. L. Efficient spacing of soil fumigants for field applications. Proc. Helminth. Soc. (Washington) 6: 62–66. 1939.
17. ————, and C. W. MCBETH. Spot treatments with chloropicrin and ethylene dichloride for control of root-knot. Proc. Helminth. Soc. (Washington) 8: 53–55. 1941.
18. YOUNG, P. A. Soil fumigation with chloropicrin and carbon bisulhpide to control tomato root knot and wilt. Phytopath. 30: 860–865. 1940.

# SOME CULTURAL PRACTICES THAT INFLUENCE THE DEVELOPMENT OF ALTERNARIA SOLANI ON TOMATO SEEDLINGS[1]

W. D. Moore and H. Rex Thomas[2]

(Accepted for publication May 28, 1943)

## INTRODUCTION

A large part of the tomato canning-crop acreage of the United States is planted with seedlings that are produced in open field beds. This type of culture has introduced some important factors such as soils, temperature, humidity, fertilization, and methods of handling and transportation, which affect both the vigor of the plants and the problem of disease control. *Alternaria solani* causes one of the most important diseases affecting tomato seedlings in the area where they are grown in open fields. Recent investigations indicate that there are several conditioning factors involved in tomato seedling production under open field conditions that, if overlooked, can materially influence the amount and severity of infection by this fungus in any given season.

The production of tomato seedlings in the South for shipment to the North has developed into a large industry, particularly important to both growers and canners. Although playing an important role in the canning industry, field production of tomato seedlings has reached its present state without much research information on the specialized cultural practices involved, particularly as they may influence the development and growth of the plant after it is set in the field. As a result of field tests conducted in the plant production area of Georgia, Hester (2) showed the importance of certain soil and fertilizer practices in growing tomato seedlings. He also suggested the inclusion of plant nutrients in the packing moss in order to maintain plant vigor during shipping. Hartman and Stair (1) made comparative studies of plants from various sources, but were interested primarily in plant growth and subsequent yields. They concluded that plant condition at the time of setting was more important than the locality from which they came. In an effort to maintain plant vigor, Sayre (4, 5, 6) found the use of nutrient "starter" solutions at the time of transplanting beneficial both in promoting rapid early growth and in the production of larger yields. Seaton and Strong (7) compared southern field-grown plants with Michigan cold-frame plants and concluded that the southern plants

[1] Conducted as a phase of cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture; the Department of Botany, Indiana Agricultural Experiment Station; the New Jersey Agricultural Experiment Station; the Georgia Coastal Plain Experiment Station; the Georgia Agricultural Experiment Station; and the Georgia State Department of Entomology.

[2] Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

were "harder" and tended to develop more disease than their own locally grown plants. During the course of tomato seedling-disease investigations, conducted by the writers in Georgia and in Indiana from 1937 to 1942, it was found that 3 factors in the production and handling of tomato seedlings especially influence the development of *Alternaria solani* on commercially grown plants. These factors are: age of plants, wilting of plants, and length of time in transit or storage. A discussion of these, together with supporting data, is presented in this paper.

## DISEASE INCIDENCE AS AFFECTED BY AGE OF PLANTS

Field-grown tomato seedlings are considered to be of the best shipping size when the height is from 6 to 9 inches. Under normal growing conditions the plants in a given field will stay within these limits for only a few days. The plants must be harvested during this critical period, or they become oversized, hard, and fibrous. Field observations have indicated that infection by *Alternaria solani* becomes appreciably greater when plantings reach this latter stage of growth, and critical buyers often refuse shipments of such plants. In order to determine the relation of plant age to *Alternaria solani* incidence, successive plantings of treated Marglobe tomato seed were made each year from 1939 to 1942. Dates of planting were as follows: 1939, March 8, 18, 28; 1940 and 1941, March 10, 20, 30; and 1942, March 11, 23, and April 2. Plots $25 \times 50$ feet were used for each planting, the rate of fertilizer application, number of rows, and rate of seeding being the same in all cases. In order that the disease might develop normally, no fungicidal sprays or dusts were used on the seedlings. As the plants in each planting reached commercial shipping size, 5 random samples of 25 plants each were pulled and packed according to commercial practice. Precautions were taken at each harvesting to pull only plants of acceptable commercial size and quality. After a storage period of 48 hours, the plants were transplanted to plots in another section of the field. This was repeated at 4- to 10-day intervals until 3 harvests had been made on each planting. After a growth period of 14 to 18 days, each transplanting was pulled, washed, and the total number of stem cankers per sample of 25 plants was recorded. Due to a lack of canker development in all plots in 1939, only leaf-spot data were taken, the readings being made in the field before pulling the seedlings. The experiments in which the plants were stored and then transplanted were conducted from 1940 to 1942.

The combined stem-canker and leaf-spot data for the 3 harvest dates during the period of these experiments are shown in table 1. Due to varying humidity conditions between both plantings and harvests, the data are not consistent in all cases. The plants did, however, tend to increase in susceptibility to infection as they became older. Of particular importance is the fact that this relationship persists regardless of the date of seeding.

Whether this increase in infection with the advance in seasonal growth was due to increased plant susceptibility, to accumulated inoculum, or to a

TABLE 1.—*Relation of age of tomato plants to infection by Alternaria solani. Mean readings of infection per (composite) sample lot of 25 tomato seedlings. Harvested at different dates from each of three separate plantings*[a] *in the years shown, and grown for 14 to 18 days as transplants*

1939

| Plantings | Harvest dates | | | | | Required difference for significance[d] |
|---|---|---|---|---|---|---|
| | 5/4 | 5/8 | 5/16 | 5/23 | 5/30 | |
| 1 | 21.4[b] | 21.4 | 21.4 | ..... | ..... | 3.8 |
| 2 | ..... | 13.0 | 19.6 | 20.8 | ..... | |
| 3 | ..... | ..... | 14.6 | 16.6 | 15.0 | |

1940

| Plantings | Harvest dates | | | | | Required difference for significance[d] |
|---|---|---|---|---|---|---|
| | 5/9 | 5/14 | 5/20 | 5/26 | 6/1 | |
| 1 | 17.4[c] | 56.4 | 50.6 | ..... | ..... | 31.2 |
| 2 | ..... | 35.2 | 31.0 | 48.8 | ..... | |
| 3 | ..... | ..... | 23.2 | 39.2 | 73.4 | |

1941

| Plantings | Harvest dates | | | | | Required difference for significance[d] |
|---|---|---|---|---|---|---|
| | 4/28 | 5/7 | 5/17 | 5/24 | 5/31 | |
| 1 | 5.4[c] | 2.8 | 57.2 | ..... | ..... | 56.6 |
| 2 | ..... | 15.0 | 44.8 | 163.4 | ..... | |
| 3 | ..... | ..... | 32.6 | 97.8 | 91.4 | |

1942

| Plantings | Harvest dates | | | | | | Required difference for significance[d] |
|---|---|---|---|---|---|---|---|
| | 5/4 | 5/7 | 5/14 | 5/16 | 5/19 | 5/26 | |
| 1 | 17.6[c] | ..... | 49.2 | ..... | 27.6 | ..... | 21.4 |
| 2 | ..... | 6.2 | ..... | 5.8 | ..... | 42.2 | |
| 3 | ..... | ..... | 17.6 | ..... | 23.0 | 62.0 | |

[a] Plantings made in 1939 3/8, 3/18, 3/28; in 1940 and 1941 3/10, 3/20, 3/30; in 1942 3/11, 3/23, 4/2.

[b] Data for 1939 show number of plants infected with leaf spot just before the harvest date.

[c] Number of stem-canker lesions per plant after storage, transplanting, and growth in the field.

[d] At the 1 per cent level.

combination of the two has not been fully determined. Spore traps failed to show an increase in air-borne spores until very late in the season; however, this does not preclude the possibility of an accumulation of spores on individual plants and a consequent rise in infection during the season. Of particular interest is the fact that infection may increase because the tomato plants have become more susceptible to *Alternaria solani* infection through a lowering of the nutritional level. This is suggested by data compiled in 1940–41 and shown in table 2 where equal lots of plants that had received 1, 2, and 3 applications, respectively, of a 4–8–4 fertilizer at 10-day intervals

were inoculated with a spore suspension of *Alternaria solani*. Following inoculation, the plants were stored for 48 hours at temperatures of 70 to 80° F., then transplanted to soil flats in the greenhouse. After growth periods of from 8 to 14 days disease readings were made. It is apparent from these data that the plants became more susceptible as the amount of available plant food was lowered.

TABLE 2.—*Means of Alternaria solani stem lesions or leaf spots on composite sample lots of 50 tomato seedlings grown in soil flats receiving different numbers of applications of a 4–8–4 fertilizer prior to inoculation, storage, and transplanting*

| Year | Experiment number | One application | Two applications | Three applications | Required difference for significance[a] |
|---|---|---|---|---|---|
| 1940 | 1 | 41.3[b] | 13.6 | 5.3 | 3.6 |
| 1940 | 2 | 216.6[b] | 158.3 | 150.7 | 6.1 |
| 1940 | 3 | 122.8[c] | 68.5 | 31.0 | 5.6 |
| 1941 | 4 | 320.0[b] | 340.2 | 192.2 | 9.8 |
| 1941 | 5 | 137.8[b] | 115.1 | 17.5 | 5.9 |

[a] At the 1 per cent level.
[b] Stem canker.
[c] Leaf spot.

The average soils in the South that are normally used for seedling production are fairly low in plant nutrients; also, fertilizer applications on these soils are held to a minimum in order to prevent too succulent growth during periods of excessive rain and when the seedlings reach shipping size. In the light of the data presented in table 2, it is possible that a part of the increased field infection is a result of these conditions, in causing a lowered physical vigor of the tomato seedlings as they grow older.

The immediate application of these findings to the requirements of the various canning areas of the North becomes strikingly evident. Due to such factors as varieties, weather conditions in the growing areas, and transportation, buyers quite often wish to draw plants from a given field for several weeks, thus causing them to become over-aged and more susceptible to infection. The above results indicate that this is a very undesirable practice.

## INFLUENCE OF WILTING ON SUSCEPTIBILITY TO INFECTION BY ALTERNARIA SOLANI

Because of the large volume of tomato seedlings the average commercial grower is forced to handle during the very short spring shipping season, instances of poor handling and packing become commonplace in most sheds. One of the most serious of these errors is that of allowing excessive wilting of plants that are not packed promptly after pulling. The average elapsed time between pulling and final packing is about 8 hours, although periods of 24 hours are not unusual. With fairly high temperatures and medium relative humidity prevailing in the packing sheds at all times, it is obvious that there is a considerable physical deterioration from evaporation and respiration during this period, although the plants freshen again after pack-

ing. Observations of disease incidence due to certain types of plant injuries (3) suggested the possibility of a relationship between plant wilting and infection by *Alternaria solani*. Accordingly, a series of experiments on this point was started in the spring of 1939 and continued through 1942.

Tomato seedlings of uniform size and vigor were pulled in bundles of 50 and treated as follows: 5 bundles were packed immediately with wet moss and wrapped with paper; a second lot of 5 bundles was allowed to remain on the table in the packing shed for 6 hours, after which the bundles were packed and wrapped as above; a third lot of 5 bundles was allowed to remain in the shed for 12 hours before packing; and a fourth lot of 5 bundles was held for 24 hours before being packed. After packing, all bundles were placed immediately in baskets and held in storage at room temperature until each lot had been stored for 48 hours. Following this period, each lot of plants was transplanted to the field. The different lots were planted in blocks of randomized rows, the blocks being replicated 5 times. No artificial inoculations were used, the inoculum coming entirely from such natural sources as are present in the average tomato field. After growing for 14 to 18 days, each replicate was pulled separately, washed, and the total number of stem lesions recorded for the 250 plants of each treatment. This procedure was followed in 2 experiments conducted in 1939, and in additional tests in 1940 and 1942.

The data for the 4 experiments are given in table 3 and also are shown

TABLE 3.—*Means of Alternaria solani stem lesion readings in four tomato experiments where samples of 250 seedlings in five replicates were subjected to varying periods of wilting prior to packing and storage*

| Date | Mean number stem cankers per 250 seedlings | | | | Required difference for significance[a] |
|---|---|---|---|---|---|
| | No wilting | 6-hour wilting | 12-hour wilting | 24-hour wilting | |
| June 1939 ..... | 201.8 | 342.0 | 404.6 | 474.0 | 8.7 |
| Oct. 1939 ..... | 115.6 | 178.6 | 288.2 | 313.8 | 5.9 |
| May 1940 ..... | 278.8 | 369.2 | 429.3 | 473.7 | 8.5 |
| June 1942 ..... | 188.4 | 233.6 | 313.8 | 299.6 | 10.4 |

[a] At the 1 per cent level.

graphically in figure 1. It is apparent that tomato seedling susceptibility increased as the period of wilting increased. The amount of disease increased significantly from the short- to the long-time periods in all of these experiments. From the standpoint of both the grower and the buyer the practical importance of these findings is apparent.

In order to further verify the role of wilting in these experiments, 2 other tests were made in which the plants were pulled and reset immediately in medium dry soil in plots replicated as described above, but the plants were watered at setting as follows: first lot, no water; second lot, 50 cc. per plant; third lot, 100 cc. per plant; fourth lot, 200 cc. per plant; fifth lot, 500 cc.

per plant. An appreciable amount of wilting occurred on the lots receiving no water, 50 cc., and 100 cc., and progressively less amounts on the lots receiving larger quantities of water. After periods of from 14 to 16 days, each replicate was pulled separately, washed, and the total number of stem lesions recorded. Data for the 2 experiments are shown in table 4. From

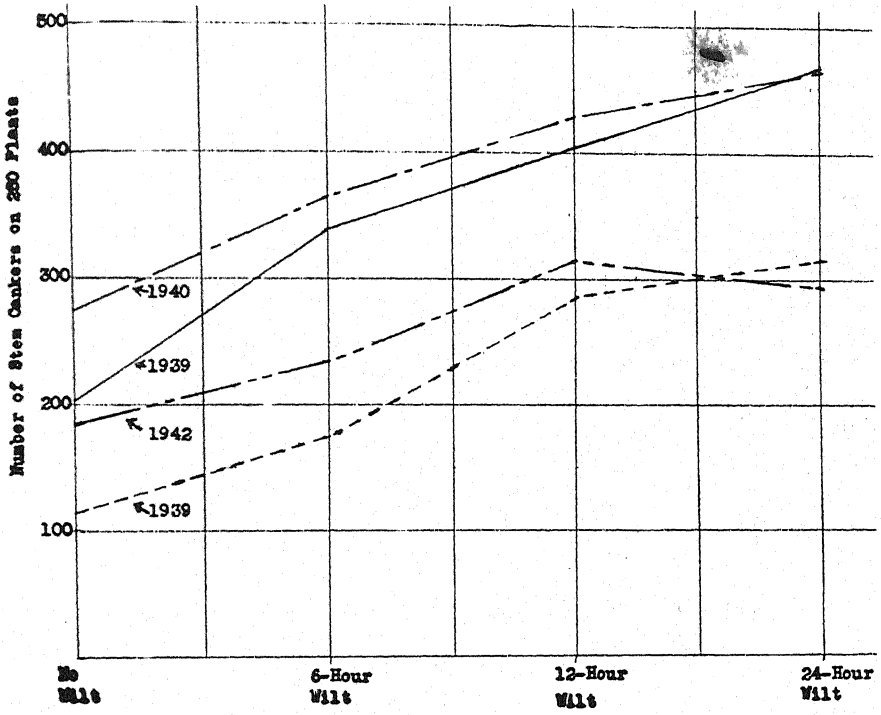



FIG. 1. *Alternaria solani* infection on lots of 250 tomato seedlings subjected to different periods of wilting before packing and storage.

TABLE 4.—*Means of Alternaria solani stem lesion readings for 150 plants in tomato seedling experiments where varying amounts of water were applied to the plants at time of transplanting*

| Year | Experiment | Cc. of water per plant | | | | | Required difference for significance[a] |
|---|---|---|---|---|---|---|---|
| | | No water | 50 | 100 | 200 | 500 | |
| 1939 | 1 | 214.0 | 118.4 | 78.0 | 80.2 | 40.6 | 6.7 |
| 1939 | 2 | 98.3 | 98.1 | 89.6 | 72.5 | 35.5 | 5.1 |

[a] At the 1 per cent level.

these data it is evident that stem-canker development decreased as the amount of water per plant was increased. Apparently, high disease incidence in these experiments was associated with wilting in the same manner as was shown in the first series of wilt tests.

## THE RELATION OF LENGTH OF STORAGE TO INFECTION BY ALTERNARIA SOLANI

The main portion of tomato plants produced in the South go to the canning areas of the North, and consequently require a shipping period of 24 to 60 hours. In many instances plants cannot be transplanted for a day or two after they are received at their destination. Since rather high temperature and high humidity exist in the baskets or crates in which the seedlings are packed, any prolonged lapse of time between time of shipment and final transplanting is likely to influence disease development. During the course of nutrient studies on tomato seedlings during the seasons of 1939, 1941, and 1942, a number of storage tests were conducted in which it was possible to make disease comparisons between lots of plants subjected to 2-day and 4-day storage periods. Lots of 5 bundles of 25 plants were packed according to commercial practice, then stored at room temperature. At the end of a 2-day period one lot was removed and transplanted to the field and another was similarly treated at the end of a 4-day period. All plantings were allowed to grow for periods of 14 to 18 days, after which each lot was pulled separately, washed, and the stem lesions recorded. The combined data for 7 of these storage tests conducted at Tifton, Ga., are shown in table 5.

TABLE 5.—*Mean number of Alternaria solani stem lesions found on lots of 50 tomato seedlings subjected to different periods of storage prior to transplanting*

| Date | Number replicates | 2-day storage | 4-day storage |
|---|---|---|---|
| June 1939 | 6 | 157.5 ± 14.3[a] | 229.1 ± 2.9 |
| June 1939 | 5 | 43.0 ± 7.4 | 67.0 ± 6.6 |
| June 1939 | 5 | 127.2 ± 13.4 | 259.2 ± 7.8 |
| June 1941 | 5[b] | 128.4 ± 17.3 | 213.6 ± 20.0 |
| June 1941 | 5[b] | 371.6 ± 34.8 | 691.2 ± 16.1 |
| June 1942 | 5 | 1368.6 ± 116.3 | 1601.0 ± 166.8 |
| June 1942 | 5 | 708.6 ± 36.2 | 1212.8 ± 126.0 |
| Totals ..... | ..... | 407.8 ± 70.2 | 599.9 ± 64.3 |

[a] Standard error of the mean.
[b] 3-day and 6-day storage.

These data indicate that infection by *Alternaria solani* increased significantly when the storage was extended from 2 to 4 days.

In 1938 a test was conducted with locally grown tomato seedlings that were inoculated with a suspension of *Alternaria solani* spores, packed in peat moss, then stored at room temperature (70° to 75° F.). After the desired periods of storage, the plants were transplanted to greenhouse benches. Stem-lesion data (Table 6) were recorded after 7 days.

Storage tests similar to the above also were conducted in Indiana in 1939, 1940, and 1942. Tomato seedlings grown in Georgia were packed as previously described and shipped by express to Lafayette, Ind. Upon arrival one lot was set in the field immediately and another held in storage for 2 or 3 days before setting. Since the normal shipping time between

Georgia and Indiana is 2 days, plants set immediately upon arrival were considered as having had 2 days of storage. The number of stem lesions or leaf spots was recorded after growth periods that varied in the different years from 11 to 27 days after setting. The data from these experiments are also given in table 6.

TABLE 6.—*Mean number of Alternaria solani stem lesions or leaf spots found on lots of 16 tomato seedlings subject to different periods of storage prior to transplanting in Indiana*

| Date | Short storage ave. number per plant | Long storage ave. number per plant | Required difference for significance[e] |
|---|---|---|---|
| May 1938[a] | 0.3 | 2.4 | 0.8 |
| June 1939[b] | 0.1 | 1.6 | 0.6 |
| June 1940[c] | 0.3 | 0.2 | .....[f] |
| June 1942[d] | 0.9 | 1.5 | 0.7[f] |

[a] Stem lesions after 1 and 3 days storage.
[b] Leaf spots after 2 and 4 days storage.
[c] Stem lesions after 3 and 6 days storage.
[d] Stem lesions after 2 and 5 days storage.
[e] At the 1 per cent level.
[f] F value not significant.

From the data shown in table 6, it is apparent that in 1938 and 1939 *Alternaria solani* infection increased on the tomato plants stored for the longer period; but in 1940 and 1942 there was no significant difference between storage periods. It should be noted, however, that 1940 and 1942 were seasons in which disease incidence was very low on all seedlings shipped from Georgia.

## DISCUSSION

The commercial production of tomato seedlings in the South and the problem of transporting them to northern canning areas is a complicated agricultural enterprise. Consideration must be given to such factors as plant nutrition, horticultural practices, weather conditions, diseases, and transportation. Whether acting individually or collectively, these several influences can affect both the general production problem and the specific problem of infection by *Alternaria solani*. Recognition of the interrelationships of these several factors is necessary in attempting to develop a system of control for this disease. The fact that increasing age of plants was found associated with increasing susceptibility to infection by *Alternaria solani*, as shown by the data in table 1, is important as only one factor among the several that are involved in the whole disease problem. Some canning areas of the North demand plants early in the spring, while others want them late. By making interval plantings in the South to meet the requirements of the respective areas of the North one phase of disease control that appears to have appreciable merit may be instituted.

While some undesirable circumstances involved in tomato plant production can be entirely removed, others can be modified or improved only to a degree. Since plant wilting cannot be entirely prevented, some damage

from this cause appears unavoidable. However, the fact that prolonged periods of wilting increased the infection by *Alternaria solani* indicates that appreciable reductions in disease losses may be possible through adjustments of field and packing-shed practices so that wilting periods will be reduced to the shortest possible time.

Time lapse in tomato-plant transportation between any two points is fairly well fixed by train schedules; consequently, holding periods for any particular shipment cannot be reduced below this minimum. However, storage of plants for additional periods of time after arrival in the North appears, on the basis of the data in tables 5 and 6, to be an undesirable practice. *Alternaria solani* is known to develop rather rapidly under conditions of high humidity and temperature and, since the average tomato plant shipment is subjected to such an environment, it is reasonable to expect increased damage from unduly extended shipping and storage periods, particularly since the present experiments indicate that during most seasons there is sufficient inoculum on the tomato plants for heavy natural infection. Experiments under way at the present time in cooperation with E. V. Miller of the Methods of Handling, Transportation and Storage section of the Division of Fruit and Vegetable Crops and Diseases, suggests that plants may be shipped successfully in refrigerator cars where optimum temperatures can be maintained by the limited use of ice in the bunkers, thus eliminating much of this damage.

## CONCLUSIONS

Amount of infection of tomato seedlings by *Alternaria solani* increases with age of the seedlings.

Susceptibility of tomato seedlings to infection by *Alternaria solani* increases as periods of wilting are increased.

*Alternaria solani* infection of tomato seedlings increases as the period of storage is lengthened.

Substantial reductions in losses or damage of southern-grown tomato seedlings, from *Alternaria solani,* can be brought about by avoiding too early planting or use of over-age plants, by prompt and careful packing to avoid wilting and mechanical injury, and by transporting and transplanting with a minimum of delay.

## LITERATURE CITED

1. HARTMAN, J. D., and E. C. STAIR. Southern- versus northern-grown tomato plants. Amer. Soc. Hort. Sci. Proc. 35 (1938): 682–685. 1938.
2. HESTER, J. B. Tomato plant production in the South. Amer. Fert. 94: 5–8; 95: 9–11. 1941.
3. MOORE, W. D. Some factors affecting the infection of tomato seedlings by *Alternaria solani.* Phytopath. 32: 399–403. 1942.
4. SAYRE, CHARLES B. Use of nutrient solutions and hormones in the water for transplanting tomatoes and their effect on earliness and total yields. Amer. Soc. for Hort. Sci. Proc. 36 (1938): 734–736. 1939.
5. ———. One and a half tons of tomatoes from fertilizer costing 48 cents. Farm Res. 5: 1 and 5. 1939.
6. ———. Starter solutions for tomatoes. Farm Res. 6: 12. 1940.
7. SEATON, H. L., and M. C. STRONG. Southern-grown vs. locally grown tomato plants. Mich. Agr. Exp. Stat. Quar. Bull. 20: 131. 1938.

# EFFECT OF MINERAL NUTRITION ON FLOWER PRODUCTION OF OWN-ROOTED ROSES AND THE INCIDENCE OF BLACK-SPOT

E. O. MADER

(Accepted for publication April 20, 1943)

In a nutrition experiment comparing grafted, budded, and own-rooted roses it was found that plants of the same variety receiving solutions of various composition responded differently to black-spot infection. This experiment was a project of the research department, Yoder Bros., Barberton, Ohio. The findings with own-rooted roses grown under greenhouse conditions and the results of studies on outdoor roses will be included in this paper. The latter were obtained from the writer's rose garden at Doylestown, Ohio.

## THE EFFECT OF MINERAL NUTRITION ON OWN-ROOTED ROSES GROWN UNDER GREENHOUSE CONDITIONS

The experiment was planned to study the effect of nutrient solutions of different composition on flower production, blindness, and bullhead formation. Gravel culture units containing Haydite as the growing medium were used. Equal numbers of young rooted rose cuttings were planted on November 18, 1938, in 6 independent gravel units. The plant material consisted of the following varieties: Briarcliff, Better Times, Johanna Hill, Ellen, and Hollywood. In the course of the investigations 4 gravel units were discontinued in April, 1939, leaving the 2 units for flower production. These units were in the center of the greenhouse and similarly located in regard to its construction.

The following nutrient solutions were applied to the plants:

| *Withrow E–2* | | | | | |
|---|---|---|---|---|---|
| Salts | $K_2SO_4$ | $CaH_4(PO_4)_2 \cdot H_2O$ | $Ca(NO_3)_2$ | $NH_4NO_3$ | $MgSO_4 \cdot 7H_2O$ |
| Purity of salts | 95 per cent | Monsanto | 82 per cent | 90 per cent | |
| Millimolar concentration | 6.0 | 0.5 | 4.0 | 4.0 | 0.5 |
| *Mader R–1* | | | | | |
| Salts | $KNO_3$ | $CaH_4(PO_4)_2 \cdot H_2O$ | $Ca(NO_3)_2$ | $NH_4NO_3$ | $MgSO_4 \cdot 7H_2O$ |
| Purity of salts | 90 per cent | Monsanto | 82 per cent | 90 per cent | |
| Millimolar concentration | 9.0 | 2.0 | 5.3 | 8.0 | 4.0 |

Solution Withrow E–2 was recommended by R. B. Withrow of Purdue University, Lafayette, Indiana, as "a solution for use during bright weather" and suitable for conditions under which the experiments were planned. Solution Mader R–1 was selected from a series of solutions under investigation, and was found most suitable for rose production under the conditions of these experiments.

The solutions were changed every 3 weeks, and the culture medium completely and repeatedly flushed with water in order to remove any excess salt.

The pH of the solutions was kept at 6.5 by daily adjustment. To these solutions the following minor elements were added: iron, 7.5 p.p.m. weekly; boron 0.5 p.p.m.; copper and zinc 0.05 p.p.m. and manganese 1 p.p.m. every 3 weeks. The units were subirrigated and 100 gal. of solution to 66 square feet of bench space were used. A 4-hour interval of automatic subirrigation was maintained throughout the experiment. In order to prevent the growth of algae on the surface of the medium the solution was pumped to within 1 inch of the top layer only. The young plants were constantly pinched (soft-pinched) until May 15, 1939, in order to build up the plant structure. Starting July 1, 1939, a production record of flowers and bullheads was kept (Table 1).

TABLE 1.—*Cut-flower and bullhead record July 1 to Nov. 30, 1939*

| Variety | Number of plants | Cut-flowers | | Bullheads | |
|---|---|---|---|---|---|
| | | Total number | Average per plant | Total number | Average per plant |
| *Solution Withrow E–2* | | | | | |
| Briarcliff | 24 | 194 | 8.08 | 15 | 0.63 |
| Better Times | 24 | 201 | 8.38 | 11 | 0.46 |
| Johanna Hill | 12 | 120 | 10.00 | 1 | 0.08 |
| Ellen | 12 | 78 | 6.50 | 1 | 0.08 |
| Hollywood | 18 | 78 | 4.33 | 2 | 0.11 |
| *Solution Mader R–1* | | | | | |
| Briarcliff | 24 | 340 | 14.16 | 63 | 2.63 |
| Better Times | 24 | 246 | 10.25 | 21 | 0.88 |
| Johanna Hill | 12 | 199 | 16.58 | 2 | 0.17 |
| Ellen | 12 | 188 | 15.67 | 8 | 0.67 |
| Hollywood | 18 | 200 | 11.11 | 9 | 0.50 |

The difference in the effect of the 2 nutrient solutions is evident. Solution Mader R–1 supported a more vigorous plant growth and flower production than solution Withrow E–2. Plants under the latter treatment produced a type of growth tending toward blindness which, in part, may explain the lower flower production.

Plants treated with Mader R–1 tended toward bullhead formation. The term "bullhead" designates a type of growth resulting in partly developed flowers. The bullhead usually is formed on a vigorous cane, and in most cases is associated with an over-fertilization with nitrogen, particularly $NH_4NO_3$. Although its nature is but partly understood, this abnormal physiological behavior in the rose plant seems related to its reaction to black-spot. Apparently, the more vigorous the plant the less prevalent is black-spot.

In the early part of August, 1939, a heavy storm deranged the ventilating system of the greenhouse. The accompanying rain kept the foliage of the plants wet for more than 6 hours. About 10 days later the plants appeared as if they had been subjected to mass inoculation with the black-spot fungus. Although the infection occurred by chance, the response of the plants under the 2 solution treatments was of interest. The own-rooted ones were selected

because they had been subjected to these 2 nutrient solutions throughout their development. No counts were taken on grafted and budded plants included in this experiment.

Premature leaf drop (a result of black-spot infection and of syringing for red spider) did not permit further counts of diseased leaflets. Counts were made from 4 plants of each variety, selected at random (Table 2).

TABLE 2.—*Total number of healthy and diseased leaflets, as well as the number of black-spot lesions*

(Figures are averages per plant)

| Variety | Healthy leaflets | Leaflets showing lesions | Total number of lesions |
|---|---|---|---|
| *Solution Withrow E–2* | | | |
| Briarcliff | 287 | 131 | 590 |
| Better Times | 352 | 136 | 612 |
| Johanna Hill | 293 | 193 | 885 |
| Ellen | 382 | 185 | 710 |
| Hollywood | 294 | 158 | 647 |
| *Solution Mader R–1* | | | |
| Briarcliff | 373 | 29 | 51 |
| Better Times | 441 | 22 | 41 |
| Johanna Hill | 354 | 53 | 86 |
| Ellen | 517 | 29 | 54 |
| Hollywood | 371 | 24 | 53 |

The difference in the number of diseased leaflets and black-spot lesions for the 2 sets of plants are striking. For some reason not yet clearly understood, the per cent of infection on the leaflets of the Mader plants was considerably lower.

It was noted that the black-spot lesions on the Withrow-solution plants exhibited a different pattern from those of the Mader plants. They were grayish, and their radiating margins gave them a feathery appearance. After a certain length of time they became more or less circular, black (necrotic) lesions, ranging from 1 to 2 cm. in diameter. Only a few lesions of this type could be found on the Mader plants, most of the lesions being small, well-defined, circular black spots. Some of these appeared like mere pin points. These lesions, particularly the small ones, suggest that infection occurred, but the progress of the disease was soon checked by the physiological state of the plants. Probably some of the smaller lesions were overlooked in the counts, hence the apparent difference in the number of lesions recorded on the two sets of plants.

Due to the nature of the experiment, no definite conclusions can be drawn as to the effect of mineral nutrition on the resistance-susceptibility of roses to black-spot. The results obtained are only suggestive. Although black-spot infection was incidental, the difference in its severity in the two sets of plants was striking. Plants with a tendency to bullhead formation, and exhibiting the most vigorous growth, together with the highest production of flowers, had the least black-spot (solution Mader R–1). These findings

confirm those of Gregory[1] who states "that many rose growers in Indiana believe that keeping the plant growing vigorously and steadily by the use of fertilizer and copious watering is an important factor in black-spot control."

Apparently a solution like Withrow's E–2, if used under conditions similar to those under which these experiments were conducted, helps to determine why the resistance of the plant is not being built up.

As nutrient solutions in gravel culture units are kept in a closed system for 3 to 4 weeks, and as there is an unequal absorption of the nutrients by the plant, the remaining salts and their combinations probably influence its physiological balance, which in turn governs the intake of ions and thus influences the type of plant growth. This assumption is supported by analyses of the two sets of plants. Differences in protein composition, as well as rate of formation, are indicated (incomplete data). It is suggested that the difference in the reaction of the rose to black-spot will be determined by the ability of the plant to obtain from the solution the necessary nutrients.

### THE EFFECT OF MINERAL NUTRITION ON OUTDOOR ROSES

The question naturally arises as to whether or not it is possible to extend the findings of the greenhouse studies to outdoor roses, particularly in regard to mineral nutrition and control of black-spot.

In the spring of 1942 a trial experiment on feeding outdoor roses entirely with inorganic salts was conducted. The garden is located at Doylestown, Ohio, and a description of its site was given by Massey.[2] The soil comprising the rose beds was a tile-drained mixture of one-half subsoil (loam) and one-half field soil (sandy loam). In the spring of 1942, 540 plants of the varieties Briarcliff, Johanna Hill, Better Times, Pink Delight, Yellow Gloria and Chieftan were set out.

### The Outdoor Rose Solution

Shortly after the indoor black-spot studies (1938–1939), the solution Mader R–1 was modified. Additional rose-nutrition experiments (unpublished) showed that by reducing the total nitrogen, particularly $NH_4NO_3$ in the solution, bullhead formation dropped and the total flower production increased. Unavailability of $Ca(NO_3)_2$ made it necessary to change to $KNO_3$ as the main source of nitrogen. The composition of the solution is as follows:

*Mader R–2*

| Salts | $KNO_3$ | $CaH_4(PO_4)_2 \cdot H_2O$ | $CaSO_4$ | $(NH_4)2SO_4$ | $MgSO_4 \cdot 7H_2O$ |
|---|---|---|---|---|---|
| Purity of salts | 95 per cent | Monsanto | | 95 per cent | |
| Millimolar concentration | 15.5 | 2.0 | 3.5 | 1.4 | 3.5 |

[1] Gregory, C. T. Fertility and the black-spot of roses. Better Crops with Plant Food 173: 17, 56–57. 1931.

[2] Massey, L. M. Diseases as influenced by garden site. Amer. Rose Magazine 4: 293–294. 1942.

The same concentration was used for feeding outdoor roses, but instead of using 100 gallons of such solution for 66 square feet bench space, as for indoor roses, 100 gallons were applied to 528 square feet of outdoor bed space. By following this procedure, approximately the same nutrient level was maintained for outdoor roses in soil as for indoor roses in gravel. The feeding of outdoor roses was bi-weekly until August 1, 1942, followed by monthly feeding until September 30, 1942. No organic material or humus was added to the soil in this experiment. Water was supplied when necessary by flooding the beds.

Although the weather of the 1942 season favored black-spot, it was observed that roses under treatment remained practically free. In frequent examinations the 540 plants showed only 157 leaves infected with black-spot. The lesions revealed the same characteristic patterns as those described for indoor plants under solution treatment Mader R–1, that is, they had well-defined margins, were small, and soon turned black. Undoubtedly the selection of a garden site having a lower dew point than its surrounding area was a factor in keeping the infection low. The type of lesions present indicated that the solution used must have been responsible to some extent for the increased resistance to black-spot fungus. The roses in this outdoor experiment exhibited an excellent vigorous growth and were prolific in flower production. Further tests are needed to differentiate between the effect of garden sites and mineral nutrition on the control of black-spot.

## SUMMARY

Data here presented indicate that rose plants, fed with nutrient solutions of various compositions, responded differently to black-spot infections.

Plants with a penchant for bullhead formation and exhibiting the most vigorous growth, together with the highest flower production, had the least black-spot; whereas plants tending toward blindness, together with the lowest flower production, showed the most. There is an apparent difference in pattern of black-spot lesion. Those on the leaves of the former plants were smaller, well-defined circular black spots, while those of the latter were larger, bore radiating margins (giving them a feathery appearance), grayish, and turning black only after a certain length of time.

An attempt was made to extend the findings of the greenhouse studies to outdoor roses, particularly in regard to mineral nutrition and control of black-spot.

R. D. 1, DOYLESTOWN, OHIO.

# ERADICANT ACTION OF FUNGICIDES ON SPORES ON LIVING PLANTS

E. M. STODDARD[1] AND J. W. HEUBERGER[2]

(Accepted for publication April 15, 1943)

## INTRODUCTION

Methods of plant disease control by fungicides have been designed heretofore to protect plants against fungous infection by applying a deposit of fungicide to the plant surfaces before spores are present. The resulting control has been considered to be a measure of the protective value of the material.

In the designing of control methods on living plants little attention has been paid to evaluating the effect of fungicides on the spores of fungi that are present when the fungicide applications are made.

The present study is not a test of fungicides as such but concerns itself only with the eradicant action of fungicides on fungus spores present on living plants and the measurement of this action in terms of spore mortality and its relation to subsequent control of the fungus. For the purposes of this paper the term eradicant refers to that action of a fungicide that renders fungus spores inactive and inhibits their germination in the infection court, so that they are incapable of producing infection.

A review of the literature on disease control by fungicides showed a wide range of reports on the eradicant action of fungicides on disease organisms; the majority of the reports deal with apple scab and seed treatment. From the literature it is evident that the eradicant action of fungicides has long been recognized, and in some control programs has been put to good use. The literature reports, for the purposes of this review, will be confined to those dealing with apple scab in the conidial stage in an active state.

*Apple Scab.* In 1922 Doran[3] reported that conidia of *Venturia inaequalis* are killed when they are hit by fresh lime-sulphur, but that this antiseptic action of lime-sulphur is not lasting, since the character of the fungicide changes on weathering. Hamilton[4] reported in 1932 that sprays containing liquid lime-sulphur may have a very considerable immediate fungicidal effect at the time of application, before the spray becomes dried and decomposed, which the wettable sulphurs do not have. Later, regarding scab control, Hamilton[5] classified spray materials on the market as those

[1] Plant Pathologist, Dept. Plant Pathology and Botany, Connecticut Agricultural Experiment Station.

[2] Plant Pathologist, Crop Protection Institute, in cooperation with the Connecticut Agricultural Experiment Station.

[3] Doran, W. L. Laboratory studies of the toxicity of some sulphur fungicides. N. H. Agr. Exp. Stat. Tech. Bull. 19. 1929.

[4] Hamilton, J. M. Recent investigations on the control of apple scab in the Hudson Valley. N. Y. (Geneva) Agr. Exp. Stat. Bull. 604. 1935.

[5] Hamilton, J. M. Studies on apple scab and spray materials for its control in the Hudson Valley. N. Y. (Geneva) Agr. Exp. Stat. Tech. Bull. 227. 1935.

that are both protective and eradicative, such as lime-sulphur and, to a lesser extent, dry lime-sulphur, and those that are generally merely protective, such as wettable sulphurs, sulphur dusts, and copper sprays.

## METHODS AND MATERIALS

The general method was to spray the fungicide on infected plants, take spores from the sprayed lesions, determine the spore mortality and relate these mortality data to control. Spore mortality was determined from the percentage that failed to germinate when removed in a large volume of water. The spores may not be killed, only inhibited, and all the adhering fungicide probably did not come off in the water. However, from the standpoint of preventing infection, it matters little whether the spore was inactivated before or after it was washed or splashed to a new locality. *Uromyces caryophylinus* on carnation and *Venturia inaequalis* on apple were the fungi and plants used in this experiment.

Various organic and inorganic fungicides were used. The organic materials were tetrachloro-para-benzoquinone (Spergon), tetramethyl thiuram disulphide[6] (Japanese Beetle Spray), ferric dimethyl dithiocarbamate (Fermate), mercaptobenzothiazole (Captax), and dinitro-o-cresol dye (Elgetol). The inorganic materials were liquid lime-sulphur, wettable sulphur (Mike), copper oxychloride (Compound A), yellow cuprous oxide (Yellow Cuprocide), and Bordeaux mixture.

### Carnation Rust

*Greenhouse Spraying.* Various materials were sprayed on infected plants in a commercial greenhouse to measure their eradicant effect on the spores present. Four applications were made: Jan. 29, Feb. 6, 20, and March 13. The viability of the spores on sprayed leaves was determined at various times during the course of the experiment. Spore suspensions

TABLE 1.—*Eradicant effect of various spray materials on the urediospores of carnation rust and on the control of new leaf infections*

| Material | Per cent concentration | Per cent spore germination | | | New infections[b] |
|---|---|---|---|---|---|
| | | 2 hr. | 17 days | 35 days[a] | 35 days |
| Check | ..... | 60 | 75 | 63 | 20.6 |
| TMTD | 0.25 | 0 | 0 | 6 | 0.9 |
| Spergon | 0.25 | 18 | 11 | 8 | 1.7 |
| Fermate | 0.25 | 0 | 23 | 22 | 1.8 |
| Yellow Cuprocide | 0.25 | 16 | 43 | ..... | 7.8 |
| Captax | 0.25 | 37 | 69 | 62 | 6.4 |
| Mike Sulphur | 0.75 | 52 | 61 | 61 | 6.1 |

[a] Time of interval after fourth spray application.
[b] Above the level of infection when spraying started. 1000 leaves counted.

[6] Hereinafter designated as TMTD, for brevity.

for germination studies were prepared as follows: 30 leaves of comparable age were taken from the plants in each treatment; spores were then taken from pustules of comparable size on each of the 30 leaves for each treatment, the final concentration of spores in double-distilled water was adjusted to 5000 to 1 cc.; drops of the spore suspensions were placed on glass slides, and incubated in moist chambers at 20° C. over night; germination counts were then made, counting 600 spores for each treatment (Table 1).

Thirty-five days after the last spray application, counts were made on the number of infected leaves in each treatment above the level of infection when spraying began (Table 1).

The data on spore germination in table 1 give a measure of the direct action of the fungicides on the spores and show also that there is a direct relation between mortality of the spores and amount of new infection.

## Apple Scab

A field test was made to determine the eradicant action of various fungicides on the conidia of *Venturia inaequalis,* and to measure the action in terms of control. This test was made in such a manner that the eradicant action as measured by spore inhibition could be related to amount of infection on new leaves produced after spraying was done.

Late in April, over-wintered apple leaves were placed under young (2-year-old) McIntosh trees. Ascospore discharge occurred on May 15, 16. No further ascospore discharge occurred and scab lesions appeared on the leaves by May 27. The infections were allowed to develop unchecked for 9 days. On June 5 the trees were sprayed with various fungicides, using 2 dosages of each material to give varying levels of spore inhibition. Each dosage was applied to 4 replicated blocks of 5 trees each. On June 16, 11 days after spraying, the effect of the fungicides on mortality of the conidia was determined.

Four sprayed infected leaves were collected from each of the 5 trees in the 4 replicate blocks, making a total of 80 leaves for each dosage, from which 40 leaves were selected at random for the working sample. A $\frac{3}{16}$-inch disc was cut out of a scab lesion on each of the 40 leaves and these discs were shaken in distilled water to make a spore suspension, drops of which were incubated on glass slides for 48 hours at room temperature, after which germination counts were made (Table 2).

A record was made on July 21 (46 days after spraying) of the amount and severity of infection on the new leaves produced after spraying was done (Table 2). The new leaves were separated into 2 groups: those on side and those on top branches. This was done to determine if the possible washing by rain of spray materials from the sprayed leaves onto the unsprayed leaves had any significant effect on the amount of scab on the unsprayed leaves.

Table 2 shows that the data on infection of new leaves on both side and

top branches were in general agreement with the data on spore inhibition; the trees sprayed with materials causing the highest spore mortality had the least amount of infection on the new growth. The data show also that there was more infection on the new leaves on the side branches than on the tops of the trees, whatever the materials used. From this it is concluded that washing of spray by rain onto unsprayed foliage had very little if any effect on control, and that the amount of infection on unsprayed leaves is a direct measure of the eradicant value of the sprays as no protective action was possible.

TABLE 2.—*Effect of various fungicides on the germination of apple scab conidia and on the amount and severity of scab infection on new leaves produced after spraying*

| Material[a] | Concentration | Spore[b] germination | Infection on new leaves[c] | | | |
|---|---|---|---|---|---|---|
| | | | Side branches | | Top branches | |
| | | | Leaves scabby | Severity | Leaves scabby | Severity |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| Check | ..... | 64.0 | 74.8 | 41.0 | 30.2 | 11.1 |
| TMTD | 0.2500 | 1.5 | 48.0 | 21.0 | 19.0 | 6.8 |
| | 0.0625 | 2.0 | 56.5 | 27.5 | 18.5 | 6.0 |
| Spergon | 0.2500 | 16.0 | 61.2 | 27.5 | 21.5 | 7.3 |
| | 0.0625 | 66.0 | 67.2 | 39.5 | 27.0 | 11.3 |
| Fermate | 0.2500 | 0.0 | 39.8 | 18.4 | 15.3 | 4.3 |
| | 0.0625 | 0.0 | 56.1 | 24.0 | 19.7 | 5.9 |
| Mike Sulphur | 0.2500 | 2.5 | 58.0 | 23.8 | 20.5 | 7.0 |
| | 0.0625 | 46.0 | 75.5 | 31.8 | 36.5 | 11.4 |
| Lime-sulphur | 0.2500 | 29.5 | 56.7 | 26.0 | 15.5 | 4.9 |
| | 0.0625 | 57.0 | 77.0 | 24.8 | 25.4 | 8.8 |
| Yellow Cuprocide | 0.2500 | 0.0 | 16.0 | 3.5 | 7.5 | 1.9 |
| | 0.0625 | 0.0 | 17.5 | 6.3 | 8.0 | 2.6 |
| Elgetol | 0.2500 | 19.0 | 64.9 | 33.3 | 28.7 | 8.9 |
| | 0.0625 | 74.0 | 69.0 | 20.3 | 32.3 | 10.9 |

[a] Materials applied on June 5.
[b] 11 days after spraying.
[c] 46 days after spraying.

The material that gave the best results, Yellow Cuprocide, was also the most injurious to the foliage; but, as we were not interested in the fungicide as such, the injury is not considered important in this experiment.

It should be mentioned here that in this experiment the materials were all used at the same percentage concentrations. For this reason lime-sulphur and Mike Sulphur were used at strengths below those recommended for use in the field. Despite this, both materials still had considerable eradicant action at the concentrations used.

## DISCUSSION

These experiments have demonstrated the eradicant action of fungicides on spores and sporulating hyphae of fungi on living plants and have pro-

vided techniques for measuring such action. The apple-scab experiment was so designed as to eliminate the influence of protective action on the results.

The carnation-rust experiments provide a yardstick for measuring eradicant action on the spores themselves and on sporulating hyphae. It will be noted that the most effective materials inhibited approximately the same proportion of spores in the population at the end of 35 days as at the end of 2 hours. This indicates an eradicant action on the spore-producing hyphae themselves. If they had continued actively to produce new spores the percentage of viable spores should have increased in time. The demonstration of this latter action gives a measure of eradicant action not obtained by laboratory techniques. The relation between spore mortality and control is strikingly close, even though eradicant and protective values are not clearly separated in this experiment.

The apple-scab experiment provides 2 measures of the eradicant action of the materials on the spores: 1. Spore inhibition and 2. Prevention of new infection on unsprayed tissue. The latter was accomplished by taking the data on control from leaves that grew after the spraying and, consequently, had no protective spray. Inasmuch as the spread of conidia of *Venturia inaequalis* is very limited, being confined essentially to the individual tree, infection on the new leaves must of necessity be caused by spores produced on that same tree. It will be noted that high spore mortality resulted in less scab on the new foliage on both top and side branches. The difference in amount of infection on the unsprayed leaves of top and side branches further corroborates the effect of eradicant action of the materials used. It has been thought that a fungicide sprayed onto tree foliage may be redistributed by rain to uncovered leaves and thus prevent their infection. If so the redistribution would be expected to be more outward and downward than upward. Hence, the lower and outer branches would be better protected than the upper branches and, consequently, less heavily infected, but they were not; in fact, the reverse was true. This indicates that the spores were splashed outward and downward more effectively than the fungicide and, hence, the increase of infection on the lower and side branches. If redistribution of the fungicides were indeed a factor in the results of this experiment, it would also be expected that the materials with the highest coefficient of tenacity would give the least control because they would be splashed around least, but if we compare Yellow Cuprocide with a tenacity coefficient of 0.834 and Mike Sulphur with a tenacity coefficient of 0.35, it is found that the reverse is true; and that the fungicide with the highest tenacity coefficient gave the best control. From these facts we must conclude that on the unsprayed foliage in this experiment the redistribution of fungicides was not a factor in the control of apple scab, and the amount of infection on the unsprayed foliage is related to the eradicant action of the fungicides on the conidia of apple scab present on the foliage at the time of spraying.

SUMMARY

It is shown that a range of fungicides of varied chemical compositions (copper, sulphur, organic) have an eradicant action on the spores of fungi present on living plants when spray applications are made.

The experiments show that control of plant diseases on living plants by fungicides is due to their eradicative action on the fungi present on the plant, as well as their protective value to the plant.

Techniques are described for measuring the eradicant action of fungicides in terms of spore mortality and control.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY,
CONNECTICUT AGRICULTURAL EXPERIMENT STATION,
NEW HAVEN, CONN.

# A GRAFT TRANSMISSIBLE MOSAIC OF AMERICAN ELM

R. U. SWINGLE,[1] P. E. TILFORD,[2] AND C. F. IRISH[3]

(Accepted for publication May 31, 1943)

A disease of American elm (*Ulmus americana* L.), resembling a virosis of the mosaic type and sometimes producing a mild to moderate brooming of branches, was observed in the vicinity of Cleveland, Ohio, in 1927 and was described briefly in 1941.[4]

Since 1927, scattered American elms, showing symptoms somewhat similar to those on the Cleveland trees, have been found throughout a large portion of Ohio and in parts of Kentucky, Michigan, and New Jersey. However, in all of these locations but few diseased trees have been observed.

Symptoms of the disease vary and often occur first on the leaves of a single branch, but eventually the entire crown may become affected. Usually some leaves are normal in size, color, and texture, but others, of the same tree or on the same branch, may be abnormally large and dark-green or small, stiff, mottled, and distorted. The smaller leaves usually develop a distinct yellow-green mottle, sometimes accompanied by rugosity; but diffused, yellow mottling, especially along the midribs, also may occur (Fig. 1). In a few cases yellow or chlorotic ring-spot symptoms have been observed on leaves of normal size.

In advanced stages of elm mosaic some leaf buds may fail to expand, leaving the branches bare of foliage for considerable distances. This gives the tree the appearance of having its leaves in bunches or tufts (Fig. 2). Many times this characteristic makes it possible to detect elm mosaic suspects from a distance. Mild to moderate brooming of branches may or may not be associated with either the yellow-green or diffuse-type mottle (Fig. 3).

No discoloration of bark or wood is produced by elm mosaic, but they frequently are dry, and separate from each other with difficulty. The wood is often brittle.

None of the trees under our observation has been killed by elm mosaic. In some cases there has been a gradual decline in vigor and some branches have died, causing the tree to become unsightly and making its removal necessary.

## TRANSMISSION

In June, 1939, roots, bark patches, and branches from 3 elms in Cleveland, Ohio, which had shown mosaic symptoms for a number of years, were grafted to healthy American elms in a nursery at Wooster, Ohio. Leaf

[1] Agent, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Columbus, Ohio.

[2] Associate Pathologist, Ohio Agricultural Experiment Station, Wooster, Ohio.

[3] Arborist, The Charles F. Irish Company, Cleveland, Ohio.

[4] Swingle, R. U., P. E. Tilford, and Charles F. Irish. A transmissible mosaic of American elm. (Abstract) Phytopath. 31: 22. 1941.

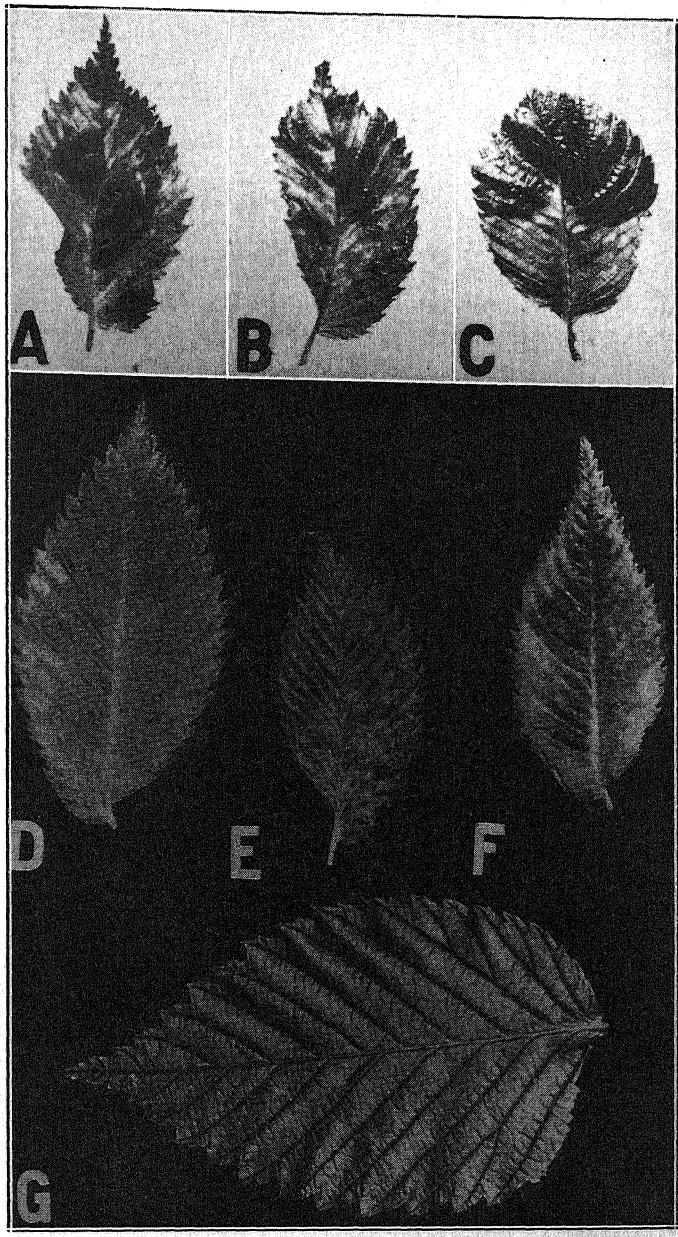



FIG. 1. A to F. Foliar mottling and distortion produced by elm mosaic. G. A healthy American elm leaf.

inoculations were attempted, using expressed sap from diseased leaves with carborundum as an abrasive. The results obtained are given in table 1.

In 1940, bark patches and buds from some of the trees successfully inocu-

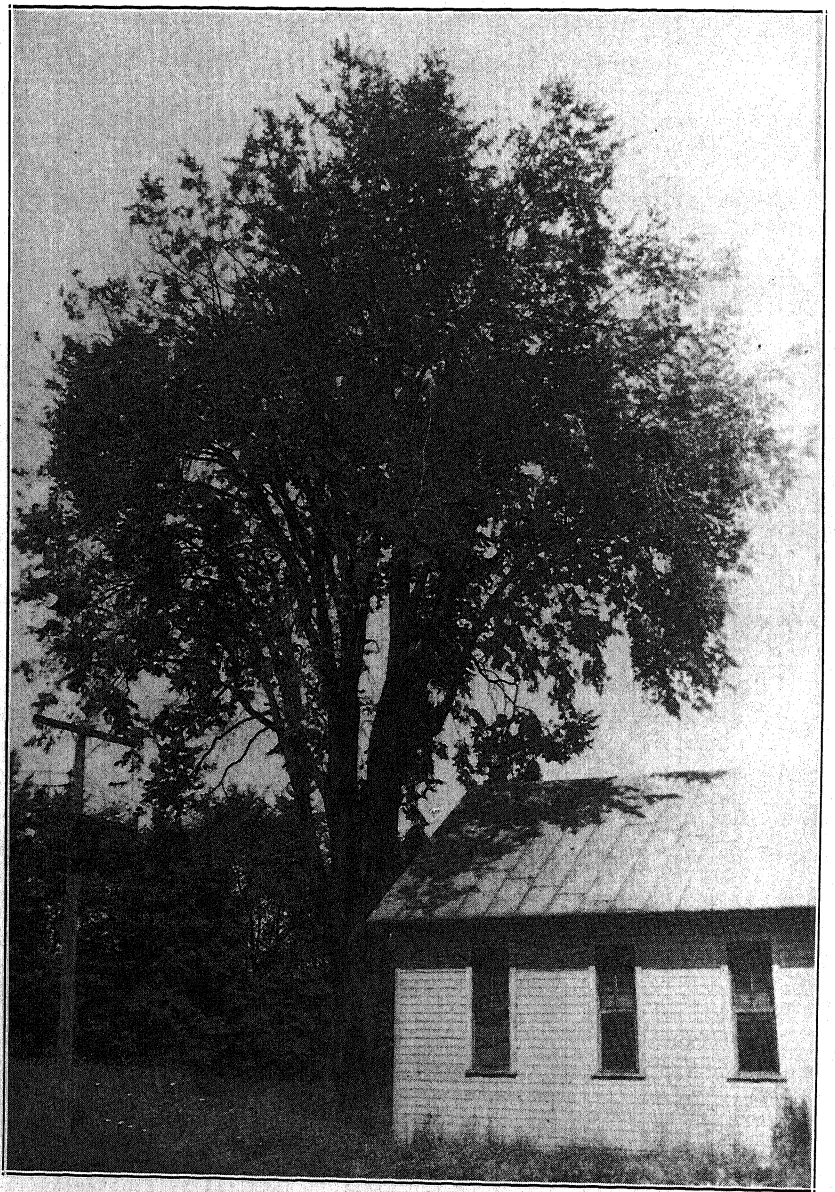


FIG. 2. An American elm affected by mosaic. Many buds fail to expand, producing symptoms of bunched and sparse foliage.

lated in 1939 were transferred to healthy trees in an attempt to retransmit the disease. Five of 6 trees grafted with diseased bark patches and 4 of 6 budded trees developed typical mosaic symptoms the following year.

Other inoculation experiments, initiated in 1940 and 1941, involved grafting over 300 healthy American elms with branch, trunk, and root material from elms with mosaic symptoms, growing in Kentucky, New Jersey,

TABLE 1.—*Transmission experiments, 1939*

| Method | No. of trees | No. of trees making graft union | No. of trees showing mosaic symptoms | |
|---|---|---|---|---|
| | | | 1940 | 1941 |
| Root splice grafts | 6 | 5 | 0 | 0 |
| Bark patch grafts | 22 | 20 | 19 | 20 |
| Branch splice grafts | 12 | 0 | 0 | 0 |
| Leaf inoculations with expressed sap using carborundum | 4 | ..... | 0 | 0 |
| Checks—grafted with bark patches from healthy elms | 3 | 3 | 0 | 0 |

FIG. 3. Brooming of branches is frequently associated with elm mosaic. Some laterals of the broom are dead.

and several localities of Ohio. So far, none of the trees grafted with root material has developed mosaic symptoms, even though, in most cases, union between stock and scion has occurred. Mosaic symptoms have been pro-

duced consistently, however, by budding and patch grafting with trunk and branch material. Usually, the symptoms produced have been of the yellow-green-mottle type, although diffuse mottle and yellow or chlorotic ring spot symptoms developed occasionally. One of the grafted trees has produced brooms.

How elm mosaic is transmitted under field conditions is not known, but there appears to be some natural spread. In Cleveland and vicinity, where mosaic elms have been under observation since 1927, apparent transmission has been slight. In some instances, healthy trees adjacent to diseased ones, sometimes with their branches intermingling, have remained healthy. In other instances, healthy trees growing near diseased ones have developed mosaic symptoms after a few years.

At Chillicothe, Ohio, 1 mosaic elm was found in 1938 in a group of trees growing in a cemetery. In 1941, 6 additional elms nearby developed typical mosaic symptoms. Later, some of these trees also developed symptoms of elm-phloem necrosis[5] and died soon thereafter.

A few other trees affected by both elm mosaic and elm-phloem necrosis have been found during investigations of the latter disease. In some of these trees, symptoms of phloem necrosis only were evident, but upon grafting healthy trees with bark patches from them, symptoms of both elm mosaic and phloem necrosis were obtained.

## CONTROL

No control measures for elm mosaic are known. A number of diseased trees have been fertilized over 2- and 3-year periods by the Aero-Fertil Method, using a 10–6–4 fertilizer. None of the fertilized diseased trees has shown evidence of increased vigor. They have continued to decline, and symptoms of bunched and sparse foliage have been retained.

During the past 15 years several attempts have been made to control the disease in trees showing initial symptoms on a few branches by removing the branches bearing mottled foliage. In all of these cases, however, symptoms have continued to develop on the leaves of the remaining branches.

## CONCLUSION

A disease of American elm (*Ulmus americana*), producing mottled foliage and sometimes brooming of branches, has been transmitted by grafting. It has been concluded from symptom and transmission studies that the causal agency is a virus. Means of spread under field conditions and control measures for elm mosaic are not known.

[5] Swingle, Roger U. Phloem necrosis, a virus disease of the American elm. U. S. Dept. Agr. Cir. 640. 8 pp. 1942.

# A HITHERTO UNREPORTED DISEASE OF THE WASHINGTON PALM

LEO PINE[1]

(Accepted for publication May 12, 1943)

A previously unreported disease of the Washington palm (*Washingtonia filifera* Wendl.) was first noticed on the campus of the University of Arizona in the late fall of 1942. Investigation disclosed that the malady was widely distributed throughout the city of Tucson. Beyond that, its prevalence is not known.

## SYMPTOMS

The first symptom of the disease is a pin-head sized or even smaller, water-soaked spot (Fig. 1, *B, a*) between the veins of the leaf blade or on the petiole. On the blade the water-soaked area gradually enlarges to form a lesion (Fig. 1, *B, b*) from 10 to 15 mm. in length, parallel to and between the veins. As the lesion increases in length the middle portion dries (Fig. 1, *B, b*) leaving a progressive water-soaked margin of 3 to 5 mm. at each end. Transverse growth of the lesion is very slight and it seldom crosses a vein (Fig. 1, *C, c, d*). On the petiole, lesions (Fig. 1, *A*) are roughly circular and usually retain that form. The fusion or numerous lesions may constitute one large diseased area (Fig. 1, *F*) covering most of the upper surface of the petiole.

The natural color[2] of the leaf of *Washingtonia filifera* varies from cedar-green to calla-green. An infected leaf may vary from the normal color to a yellowish oil-green or an oil-green. The oil-yellow-green of the young water-soaked spots does not conspicuously stand out against the normal green of the leaf. As the lesion increases in size the water-soaked part changes to dark-ivy-green and the drying internal portion becomes olive-buff to fire-clay colored surrounded with the dark-ivy-green, water-soaked tissue. Eventually, on complete drying out of the lesion, the tissue takes on an olive-buff to deep-olive-buff color. The changes that take place in the lesions on the petiole parallel those in lesions of the blade, although the final stage of infection may here present a somewhat darker shade.

Economically, the leaf-spot disease of Washington palm is important only to the extent to which the spots may detract from the ornamental appearance of the leaves. The infection appears incapable of killing an entire leaf. Since the most extensive infection is limited to the older leaves of the palm, the diseased parts may be mostly removed in seasonal pruning.

[1] The author gratefully acknowledges the assistance and criticisms of Dr. J. G. Brown, Professor of Plant Pathology, and of Mrs. Alice Boyle, Research Assistant, during the progress of the work. His thanks also are due to Dr. R. B. Streets, Associate Professor of Plant Pathology, who made the photomicrographs used in this paper.

[2] All colors mentioned in this paper refer to "Color Standards and Nomenclature" by Ridgway.

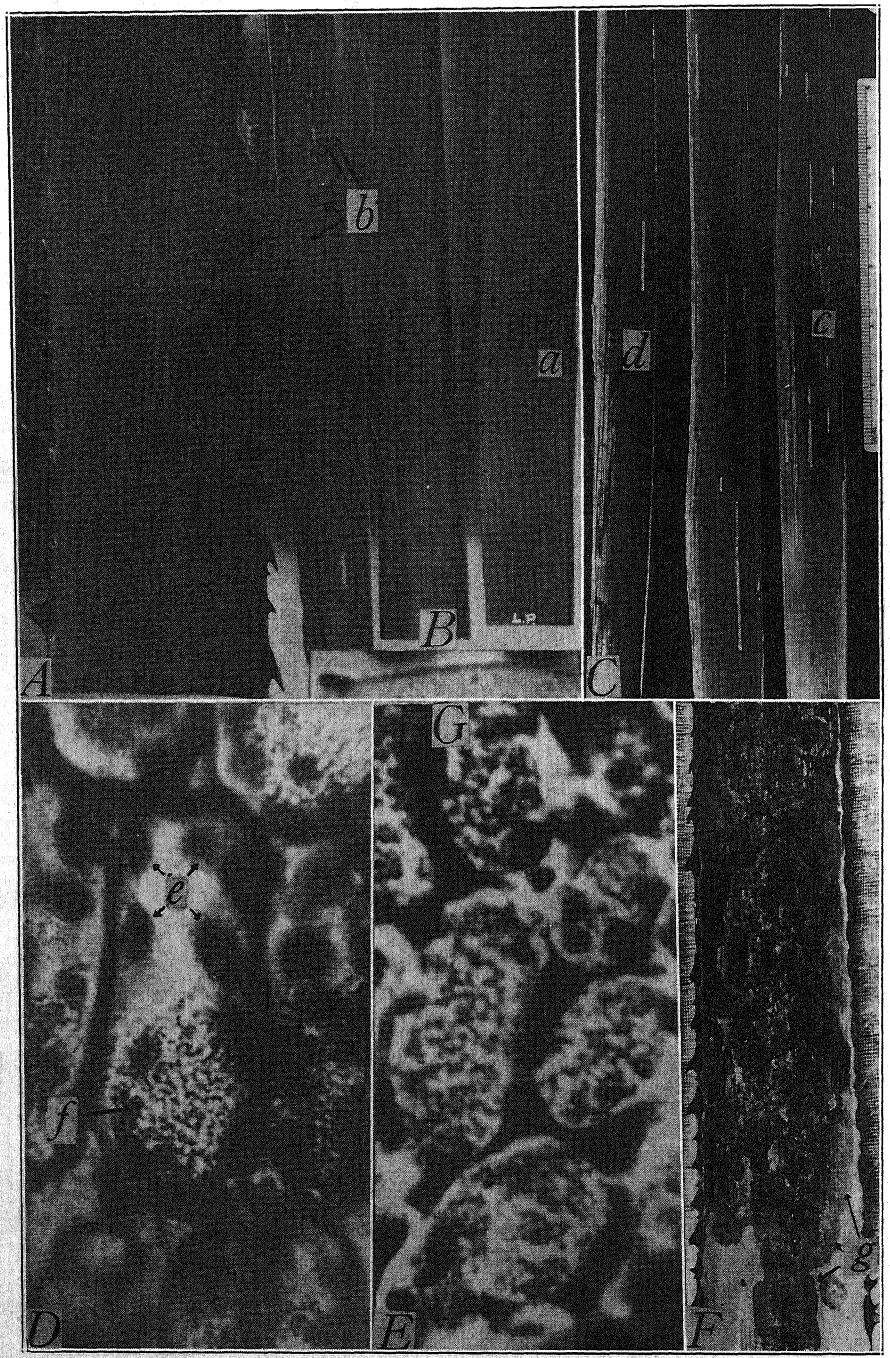



FIG. 1. *A*. Section of petiole with water-soaked lesions. *B*. Pieces of three lobes of leaf-blade showing progressive stages of lesions: *a*, numerous early stages of infection; *b*, lesions elongated between veins of leaf; the older ones with internally dry and necrotic tissues. *C*. Parts of lobes of blade with advanced linear lesions: *c*. fusion of lesions;

### ISOLATION OF THE CAUSE

Small sections of tissues containing water-soaked areas were cut from infected leaves, dipped in mercuric chloride, 1:1000, for 15 to 30 seconds, and placed on P.D.A., pH 7.0. On the plates appeared 4 different types of colonies, of which 3 were, respectively, a Gram-positive bacillus, a smaller Gram-positive rod, and a yeast. The fourth type of colony, present in the majority of cases and that appeared in almost pure culture, was a small Gram-negative rod. Because the last showed greater probability of being the primary causal organism, it was selected for study. Poured plates of crystal-violet agar (1 cc. of 1:1000 crystal violet in aqueous solution per plate of P.D.A.) gave pure cultures of the bacterium.

The isolated organism in pure culture was inoculated into campus palms that appeared to be free from infection, by spraying the inoculum on the under side of the leaf blade and also by the needle method in both petiole and blade. Seven days later inoculations of both series had taken, small water-soaked lesions appearing at the base of the angles formed by the junction of the lobes with the petiole and around needle punctures. That these artificial infections did not progress so rapidly as the natural ones was attributed to the fact that the inoculations were made in late spring, a period in which little or no natural infection has been found. Re-isolations made from the lesions resulting from inoculations gave, with few exceptions, a pure colony of a bacterium identical in appearance with that used in the inoculum. Physiological tests of the bacterium gave results that corresponded precisely with those of the organism isolated from the natural lesions.

### DESCRIPTION OF THE BACTERIUM

The cause of the leaf spot on *Washingtonia filifera* is a non-spore-forming, Gram-negative, motile, polar-flagellate (1–3 mostly uni-) rod (Fig. 1, *G*) with rounded ends, 1.61 $\mu \times 0.69$ $\mu$, occurring singly or in chains of 3–4, forming entire-margined, smooth-surfaced, convex, glistening, white to cream-colored, butyrous, circular colonies 1½ to 2 mm. in diameter (P.D.A., 10 days, 20° C.) and filiform colonies on slants of the same medium. Physiologically, the bacterium is aerobic, has a T.D.P. of 47–48° C. (bouillon, 10 min.) is gelatine-liquefying, non-starch-hydrolysing, non-indol-producing, non-nitrate-reducing, and produces $H_2S$ in very small amounts; in milk it is non-curdling, peptizing, and imparts a green color to litmus milk within 7 days. It gives an acid reaction to glucose, fructose, l-arabinose, in 24 hours; to galactose and xylose in 48 hours; no acid in sucrose, lactose, cellibiose, maltose, mannitol, d-sorbitol, glycerine, salicin, and raffinose in 6 days, and produces no gas within 6 days in any of the above carbon sources. For the bacterium is suggested the name, **Phytomonas washingtoniae.**

### EFFECT ON HOST

The conditions that seem to be most suitable for infection of the host with the leaf-spot bacterium are those of periods with rain and low tempera-

*d*, an advanced stage of fusion. *D*. Photomicrograph of longitudinal section of a parenchyma cell from water-soaked area of leaf: *e*, normal chloroplasts in cytoplasm; *f*, bacteria that have invaded base of cell through broken wall. *E*. Disintegrating parenchyma cells from the drying area of lesion on leaf blade, in which masses of bacteria completely fill cells. *F*. Lesions that started as shown in *A* and *B* have coalesced and now practically cover the entire upper surface of a long petiole; *g*, water-soaked margin of lesion. *G*, Polar, uniflagellate bacterium.

ture. Therefore, the disease occurs in late fall and winter. Upon the appearance of drier and warmer weather, the causal bacterium becomes inactive and very little, if any, new infection follows. From the results of spraying with bacterial suspensions and the appearance of normally infected leaves, it is evident that the bacterium enters the host *via* the stomata that are present in both upper and lower epidermis of the leaves. Infection more commonly occurs on the ventral side. Natural infection is present mainly on the older, more horizontal leaves that hold liquid moisture longer than the younger vertical leaves.

Lesions will penetrate the entire thickness of the blade of palm leaves, but seem to affect only shallowly the surface of the fleshy petiole; they are limited to the parenchyma (Fig. 1, *D, f; E*) tissue. That lesions seldom cross a vein in the leaf is probably due to the small amount of parenchyma between vein and epidermis.

Attempted inoculations of green and semi-ripe date fruits gave no infection. Examination of the date palms on the campus showed various lesions on petioles and leaflets, but attempts at isolating the same causal organism occurring on *Washingtonia* were negative.

## SUMMARY

A new disease has been reported for *Washingtonia filifera*. This disease, which affects the leaf of the palm, does not appear to be capable of killing the entire organ and is economically important only in so far as it alters the ornamental value of the leaves by spotting them. As yet the disease has not been observed on palms other than *Washingtonia*. The name of **Phytomonas washingtoniae** has been given to the causal organism.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF ARIZONA,
TUCSON, ARIZONA.

# INHIBITION OF PHYMATOTRICHUM SCLEROTIA FORMATION BY SULPHUR AUTOCLAVED WITH SOIL

A. A. DUNLAP

(Accepted for publication May 18, 1943)

In addition to the well-known toxic effects of sulphur on fungi, small quantities of finely-ground sulphur, thoroughly mixed and autoclaved with soil, have been found to inhibit the formation of sclerotia by the cotton root-rot fungus (*Phymatotrichum omnivorum*). Mycelial growth of the fungus did not appear to be affected by the sulphur treatment, although no thickening of the mycelial strands to form the sclerotia took place when about 1 part (or more) of sulphur was added to 2000 parts of air-dry soil. This effect of sulphur is different from the prevention of infection by *P. omnivorum* reported by Taubenhaus and Ezekiel,[1] in which case an increase in soil acidity was caused by the oxidation of sulphur by soil organisms.

The same type of sterile-soil culture as was used[2] for obtaining sclerotia of *Phymatotrichum omnivorum* in quantity were employed in this study. Air-dry Houston black clay soil (about 8 per cent moisture content on an oven-dry basis) was passed through a 20-mesh sieve and definite amounts of dusting sulphur (98 per cent passing a 325-mesh screen) were added to weighed amounts of the soil. Different brands of dusting sulphur, with and without a conditioning agent, were used with like results. After thorough shaking in a stoppered flask, 100 g. aliquots of the sulphur-treated soil were placed in 250 ml. flasks. The same amount of soil with no sulphur added was placed in other flasks for controls. After adding definite amounts (25 to 40 ml.) of tap water to the soil, 5 g. of sorghum seed were placed on the surface of the soil and the flask plugged with cotton and sterilized at 15 lb. pressure for one hour. The flasks were inoculated with a pure culture of *P. omnivorum* on agar. The cultures were allowed to grow for 5 weeks at room temperature and the sclerotia were separated by washing the soil through a 40-mesh sieve. They were then placed on filter paper for about an hour before weighing.

Table 1 shows the average fresh weights (each figure represents an average of 10 flasks) of sclerotia obtained from two series of the variously treated soil cultures and from the checks. At each of the soil-moisture levels, no sclerotia were formed when sulphur was added at the rates of 1 part per 500 or 1000 parts of soil. Only traces of sclerotia were found at the next lower concentration of sulphur (1:2000). At the 1:4000 concentrations of sulphur, about one-half of the normal amount of sclerotia were produced and at 1:8000 the amount of sclerotia produced was practically equal to that in the nontreated soil. There was little difference in the effects of the

[1] Taubenhaus, J. J., and W. N. Ezekiel. Relation of soil acidity to cotton root rot. Tex. Agr. Exp. Stat. Bull. 545. 1937.

[2] Dunlap, A. A. A convenient soil-culture method for obtaining sclerotia of the cotton root rot fungus. Amer. Jour. Bot. 28: 945–947. 1941.

sulphur between the various levels of moisture. Figure 1 shows Petri-dish cultures of the fungus—one side of each Petri dish contained soil to which sulphur was added and the other side (showing sclerotia on the bottom) was filled with nontreated soil.

The seeds on the surface of the sulphur-treated soil were covered with mycelium and completely decayed by the fungus. Mycelial strands were also seen growing close together over all surfaces of the treated soil, and hyphae were found throughout the soil mass. This condition was found to exist when as much as 1 part of sulphur to 65 parts of soil were used. Other experiments made with navy beans, corn, cowpeas, and barley as

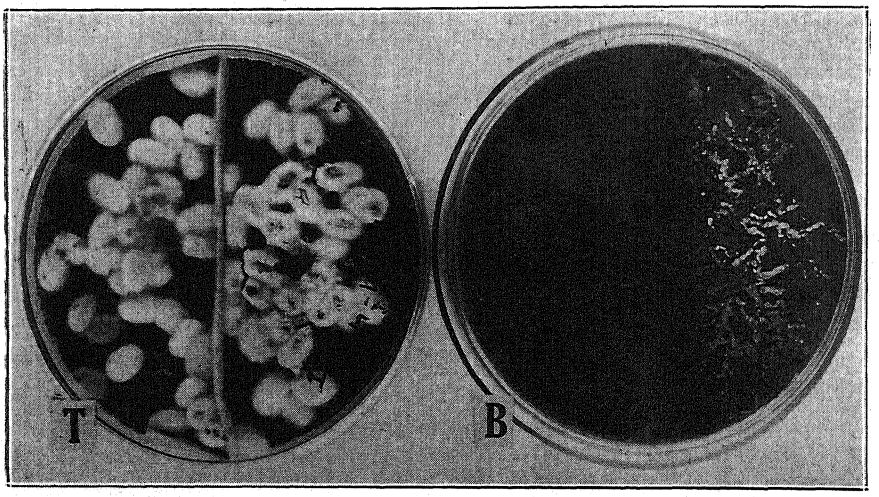



FIG. 1. Petri-dish cultures of *Phymatotrichum omnivorum* on Houston black clay soil with navy beans on the soil surface for nutrient, 20 days after inoculation. T, top of a culture; B, the bottom of a similar culture. The soil in the left half of each culture was treated before autoclaving with 325-mesh sulphur at 1: 1000; the soil in the right side (with sclerotia) was nontreated.

sources of nutrient gave results similar to those with sorghum seed. Also, no sclerotia were formed in a noncalcareous, slightly acid soil that was treated with similar quantities of sulphur and autoclaved. The addition of calcium carbonate to acid soils (5.4 to 6.1 pH) did not make the sulphur treatments with smaller amounts of sulphur any more effective.

As compared with sulphur treatments, sclerotia formation was not proportionally decreased by the addition of dry lime-sulphur (Sherwin-Williams) to the dry soil, in amounts supplying sulphur in excess of 1: 1000. Likewise, sodium sulphide (Merck, reagent), sodium polysulphide (fused, 60 per cent), potassium sulphide (Merck, Tech.), and calcium sulphide (U.S.P.) did not prevent sclerotia formation when dissolved in the water that was added to successive layers of the air-dry soil in the culture flask before autoclaving. Treatment of the soil with materials containing organic mercury compounds (Semesan, Sanoseed) and with cuprous oxide (red

TABLE 1.—*Effect of sulphur on formation of sclerotia in autoclaved soil cultures of Phymatotrichum omnivorum with varying soil moisture*

| Sulphur application | Fresh weight of sclerotia per flask | | | | |
|---|---|---|---|---|---|
| | Amount of water per flask | | | | Average |
| | 25 ml. | 30 ml. | 35 ml. | 40 ml. | |
| | *g.* | *g.* | *g.* | *g.* | *g.* |
| 1: 500 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1: 1000 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1: 2000 | 0.3 | 0.3 | 0.2 | 0.1 | 0.2 |
| 1: 4000 | 1.2 | 2.0 | 3.0 | 2.7 | 2.2 |
| 1: 8000 | 3.9 | 4.6 | 4.0 | 3.9 | 4.1 |
| None | 4.0 | 4.6 | 4.1 | 3.9 | 4.2 |

Cuprocide), in amounts similar to the quantities of sulphur used, likewise failed to prevent sclerotia formation under these conditions.

It was apparently necessary that a finely divided sulphur be used for most effective inhibition of sclerotia formation and that the sulphur be thoroughly mixed and autoclaved with the soil. This inhibition was much less marked when coarse soil sulphur (16-mesh and finer) was used. This treatment would represent a smaller sulphur dosage because of the reduced amount of particle surface as compared with dusting sulphur. Placement of the fine sulphur on the surface of the soil without mixing in the flask did not inhibit sclerotia formation. Treatment of the soil with a solution of sulphur in carbon disulphide—allowing the carbon disulphide to evaporate before placing the soil in the flask—prevented sclerotia formation and this method required as much sulphur (for equal results) as mixing the dry dusting sulphur into the soil.

The autoclaving process may have resulted in the formation of a toxic sulphur material that inhibited the sclerotial stage of the fungus. Autoclaving appears necessary because sclerotia were formed in abundance in similar soil-sulphur mixtures that were sterilized in a hot-air oven at 180–200° C. for 5 hours, with the sterile water and seeds added later. No significant differences in pH were found in the Houston black clay soil, following autoclaving with the small amounts of sulphur. A toxic compound, however, apparently was not formed from the sulphur compounds added to the soil before autoclaving. There was a distinct odor of hydrogen sulphide in the soil cultures following growth of fungus, in cases where enough sulphur was added to prevent the formation of sclerotia. In flasks sealed with Parafilm after inoculation, a reduction of aerial growth of mycelium was noted in 1:1000 sulphur-treated soil although abundant aerial growth was present in the 1:4000 flasks. (Few sclerotia are formed in sealed flasks.[2]) The hydrogen sulphide was probably formed from reduction of the sulphur by the fungus, as similar non-inoculated flasks showed no odor after 5 weeks. Wettable and colloidal sulphurs were found to

[2] Dunlap, A. A. *l.c.*

lessen the rate of growth of *Phymatotrichum omnivorum* in both liquid and agar media. In liquid cultures, small amounts of potassium or calcium sulphide prevented growth of the fungus entirely.

SUMMARY

When thoroughly mixed with the soil and autoclaved, one part of 325-mesh dusting sulphur to 1000 parts of air-dry black Houston clay soil resulted in complete inhibition of sclerotia formation by *Phymatotrichum omnivorum,* with no apparent effect on mycelial growth. Only traces of sclerotia were formed when a sulphur : soil mixture of 1 : 2000 was used and no inhibitive effects of the sulphur were noted at 1 : 8000. It was necessary to use a finely-ground sulphur and to mix the sulphur thoroughly with the soil in order to prevent sclerotia formation. The inhibitive effect was due possibly to the formation of a toxic compound upon autoclaving.

TEXAS AGRICULTURAL EXPERIMENT STATION,
COLLEGE STATION, TEXAS.

# ASSOCIATION BETWEEN NEMATOSPORA PHASEOLI AND THE GREEN STINKBUG[1]

J. G. Leach and Genevieve Clulo

(Accepted for publication May 20, 1943)

In 1940 Leach (2) reviewed the literature dealing with stigmatomycosis and its transmission by various species of plant bugs. Available published information led him to make the following statement. "The universal association of species of *Nematospora* with the insects in such widespread regions of the world would suggest that the association is more than a casual one. A thorough study of the possible symbiotic relationships between insect and fungus should be made. It is significant that no satisfactory explanation of the method of survival of the fungi over winter has been offered by any worker on the disease. The question of whether the fungus is constantly associated with the insects throughout their life history or whether they pick it up from some unknown source has never been answered."

One of the most common forms of stigmatomycosis in the United States is yeast spot of beans, caused by *Nematospora phaseoli* Wingard. Yeast spot has been studied extensively by Wingard (5) who first presented experimental proof of its transmission by the stinkbug. In regard to the method of transmission, Wingard (6) stated "These results indicate that *Nematospora* is carried by the above-named insects, but whether it is carried internally or externally remains to be determined. Nowell has attempted to isolate it from *Dysdercus delauneyi* and *Nezara viridula* but without success, and I have had the same experience with *Nezara hilaris*. Other yeasts and yeast-like fungi are very readily obtained from this insect but I have consistently failed to isolate *Nematospora phaseoli*. I am inclined, nevertheless, to believe that these insects are themselves infected with the organism. The negative results obtained thus far are very probably due to improper technique."

Stigmatomycosis appears to occur most abundantly in the tropical and subtropical regions of the world, although some forms of the disease occur in the warmer regions of the temperate zones. Yeast spot of Lima beans is common in southeastern Virginia, but has been observed only a few times in West Virginia. In the summer of 1941 a study of the association of yeast spot and the green stinkbug was undertaken with the hope of throwing some additional light on the method of transmission and possible overwintering of the pathogen within the body of the insect vector.

Although a fairly extensive survey of several localities in West Virginia was made, the disease was not found. The stinkbug (*Nezara hilaris* Say), however, was fairly prevalent on Lima beans and various noncultivated hosts. A careful search disclosed none of the disease, even when the insects were very numerous on Lima bean plants. Isolations were made from the

[1] Published with the approval of the Director, West Virginia Agricultural Experiment Station, as Scientific Paper No. 307.

feeding punctures of the bug on Lima bean pods, as well as from both internal and external organs of the stinkbugs, both nymphs and adults, but these failed to yield a culture of *Nematospora*. Several kinds of culture media were used on all of which the yeast grows well. For comparison with these specimens some stinkbugs from southeastern Virginia collected from Lima bean plants affected with yeast spot were obtained through the courtesy of Dr. S. A. Wingard. *Nematospora* was isolated readily from the surface of these insects but none was obtained from the internal organs aseptically dissected from surface-sterilized insects.

As a further check on possible internal transmission, specimens of bugs, both nymphs and adults, were imbedded in paraffin, sectioned and stained with the modified Gram-Weigert method (6). A careful search did not reveal *Nematospora* within the body of the specimens from either source. In certain individual insects microorganisms were present in the gastric caeca, but these obviously were not *Nematospora*. They were in all probability the microsymbiotes described by Glasgow (1). Efforts to grow these in artificial culture were unsuccessful. Although they were removed aseptically from freshly dissected insects and plated in agar, they did not grow.

The possibility of internal biological transmission seems unlikely when one considers the relative diameters of the yeast cells and the food channel of the insect's mouth parts. Measurements of the food channel of adult insects show that it rarely exceeds 12 μ in diameter, while the salivary channel is several μ smaller. Although some of the immature vegetative cells of *Nematospora* may be smaller, the great majority of mature cells are from 10 to 20 μ in diameter, much too large to permit ready passage.

The absence of yeast spot in association with stinkbugs on Lima beans in West Virginia indicates that the association of the disease with the stinkbugs is not a constant one, even though the disease may depend upon the insect for inoculation. The failure to find *Nematospora* within the body of insects taken from plants affected with yeast spot would indicate that transmission may be entirely external and mechanical.

Nowell (3), in his study of the internal disease of cotton bolls in the West Indies, reported 4 species of fungi transmitted by several species of bugs. All 4 of these fungi were found affecting the fruits of several species of plants, mostly legumes, that serve as hosts for the insect vectors before they migrate to the cotton bolls. The inference from Nowell's work seems to be that these weed hosts serve as sources of contamination of the bugs. Although Nowell reported no histological work with the bugs, he showed in one experiment that bugs reared on healthy plants did not transmit the fungi when allowed to feed on cotton bolls. Bugs from diseased plants transmitted the fungi readily.

Even though *Nematospora phaseoli* does not survive within the body of hibernating adult bugs, it could possibly overwinter as resistant cells or spores on the external parts of the insect. It seems more likely, however, that the bugs become contaminated in the spring by feeding on infected

material. Underhill (4) reports that Lima beans are not the preferred host of the stinkbug and that they usually migrate to beans after feeding for some time on certain preferred noncultivated plants. This possibility is supported by the greater prevalence of yeast spot in the regions of mild winters, where infected plant tissues would not be destroyed by freezing and where actively growing yeast cultures would more likely survive and be available. If the pathogen survives the winter in this way the discovery and elimination of the source of contamination might offer a practical means of control. Very little is known of the host range of *Nematospora phaseoli* in nature. This should be determined, but it can be done effectively only in milder regions where the disease is more abundant than it is in West Virginia.

WEST VIRGINIA AGRICULTURAL EXPERIMENT STATION,
MORGANTOWN, W. VA.

LITERATURE CITED

1. GLASGOW, H. The gastric caeca and caecal bacteria of the Heteroptera. Biol. Bull. 26: 101–156. 1914.
2. LEACH, J. G. Insect Transmission of Plant Diseases. McGraw-Hill Book Co. (New York). 1940.
3. NOWELL, WILLIAM. Internal diseases of cotton bolls in the West Indies. West Ind. Bull. 17: 1–28. 1918.
4. UNDERHILL, G. W. The green stink bug. Va. Agr. Exp. Stat. Bull. 294. 1934.
5. WINGARD, S. A. Yeast spot of Lima beans. Phytopath. 12: 525–532. 1922.
6. ———. Studies on the pathogenicity, morphology, and cytology of *Nematospora phaseoli*. Bull. Torr. Bot. Club 52: 249–290. 1925.

## PHYTOPATHOLOGICAL NOTES

*Occurrence of the Imperfect Stage of Sclerotinia laxa on Prunus cerasus in Wisconsin.*—On June 18, 1941, trees of the Montmorency and Early Richmond varieties of *Prunus cerasus* L. in a commercial orchard near Bailey's Harbor, Door County, Wis., were observed to be severely affected by a blossom and twig blight (Fig. 1, A). No sporulation of any known pathogen

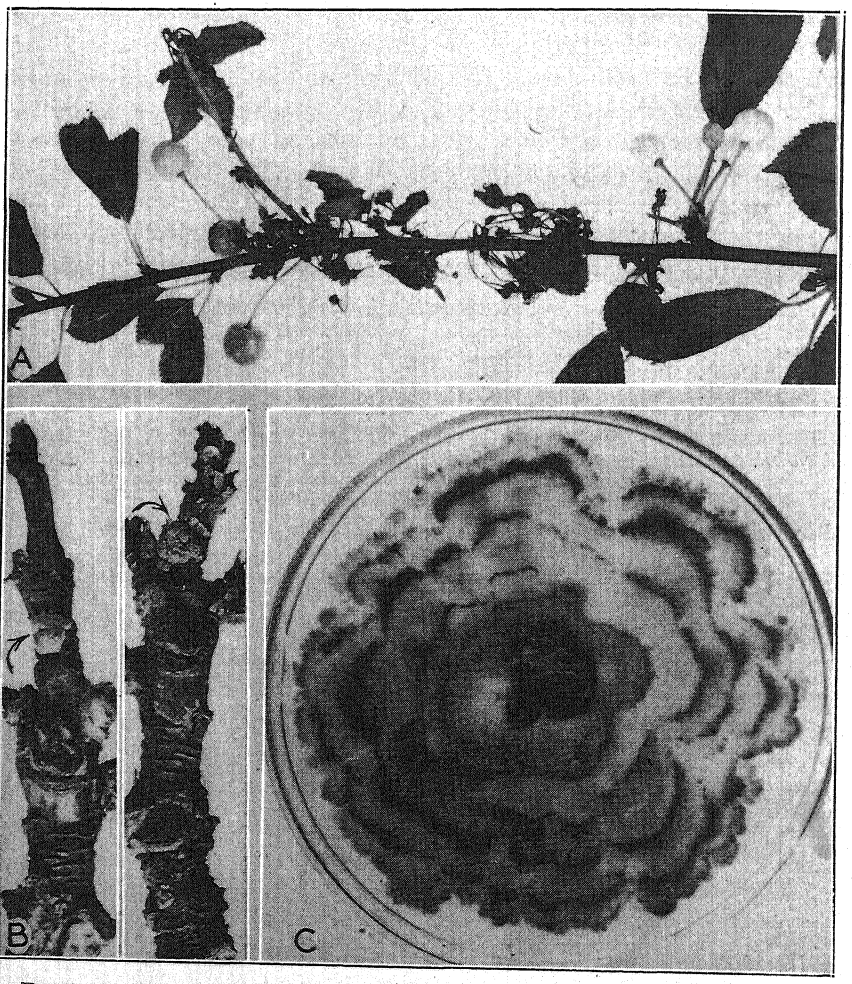



FIG. 1. A. Blossom and twig blight of Early Richmond cherry, incited in the current season by *Sclerotinia laxa*. B. Early Richmond fruit spurs blighted in the preceding year, showing sporodochia (at arrows) of *S. laxa* developed in the current spring. C. *S. laxa* on potato dextrose agar after three weeks at 24° C.

was observed on the diseased parts, but plate cultures from internal tissues of diseased twigs consistently yielded a fungus showing the cultural characters of *Sclerotinia laxa* Ader. and Ruhl.[1,2,3]

[1] Ezekiel, Walter N. Fruit-rotting Sclerotinias. II. The American brown-rot fungi. Md. Agr. Exp. Stat. Bull. 271: 87–142. 1924.

When the same trees were examined in the spring of 1942 abundant sporodochia of the fungus were found. These occurred most commonly on the blighted fruit spurs of the preceding year (Fig. 1, B), but also on the remains of petioles and midribs of the leaves and persistent parts of the overwintered blighted blossoms. A severe local outbreak of this blossom and twig blight occurred in this orchard in the spring of 1942, but no sporodochia were observed on lesions developed in the current season. However, in the spring of 1943 a few sporodochia were observed on blossoms blighted in the current year. Platings from such sporodochia yielded *Sclerotinia laxa.*

The fungus was isolated at will (Fig. 1, C) from internal tissues of blighted twigs in spring and fall. It was grown on potato dextrose agar in comparison with *Sclerotinia fructicola* (Wint.) Rehm. It was used in the greenhouse to inoculate blossoms and twigs of *Prunus cerasus* (Montmorency), on which it incited symptoms characteristic of *S. laxa.* Control plants were not diseased. The organism was reisolated from an infected plant and found to have the same cultural characters as the cultures used for the inoculations. Reinoculation from these cultures resulted in typical infection.

The Door County cherry district has not been completely surveyed for *Sclerotinia laxa,* and it is not yet possible to evaluate the danger of this pathogen to the Wisconsin cherry industry. Local outbreaks similar to the one reported have been observed in certain nearby orchards, and the fungus has been found in less conspicuous development in several other locations in the cherry district of Door County. Experiments with both eradicant and protectant sprays for the control of this brown-rot disease have been initiated.

*Sclerotinia laxa,* the common brown-rot fungus of stone fruits in Europe, has long been known to occur in Europe and the British Isles, Manchuria and Japan, and along the Pacific Coast of North America.[3] So far as the writers are informed, its occurrence in central or eastern North America has not hitherto been proved.[1,2,3]—G. W. KEITT, J. DUAIN MOORE, E. C. CALAVAN, and J. R. SHAY, University of Wisconsin, Madison, Wisconsin.

*Masking of Leaf Symptoms of Sour-cherry Yellows by Temperature Effects.*—The leaf symptoms of cherry yellows,[1,2,3,4] a virus disease, may

[2] Ezekiel, Walter N. Presence of the European brown-rot fungus in America. Phytopath. 15: 535–542. 1925.

[3] Harrison, T. H. Brown rot of fruits and associated diseases of deciduous fruit trees. I. Historical review, and critical remarks concerning taxonomy and nomenclature of the causal organisms. Jour. and Proc. Roy. Acad. N. S. Wales 67: 132–177. 1933.

[1] Keitt, G. W., and C. N. Clayton. A destructive bud-transmissible disease of sour cherry in Wisconsin. Phytopath. 29: 821–822. 1939.

[2] Rasmussen, E. J., and Donald Cation. A progress report on the yellow-leaf disease of sour cherry. Mich. State Hort. Soc. Ann. Rept. 71: 100–105. 1942.

[3] Hildebrand, E. M., G. H. Berkeley, and D. Cation. Handbook of virus diseases of stone fruits in North America. Mich. Agr. Exp. Stat. Misc. Pub., 76 pp., 1942.

[4] Keitt, G. W., and C. N. Clayton. A destructive virus disease of sour cherry. Phytopath. 33: 449–468. 1943.

vary greatly in degree of expression in different seasons or regions. The cause of these variations, which have been a substantial barrier to the progress of investigations of this disease, has not been explained. The present paper gives a preliminary report on experiments indicating that temperature is a sharply limiting factor in the expression of leaf symptoms of this disease.

In a preliminary experiment in the spring of 1942, potted Montmorency trees in which buds from Montmorency trees affected by yellows had been inserted were incubated comparatively in greenhouses in which the temperatures were approximately 16, 20, 24, and 28° C., respectively. Some of these trees were budded in the greenhouse in January; others had been brought in a dormant condition from the field, where they had been budded and had shown yellows symptoms. The leaf symptoms of yellows developed freely in the 16° house. Only one tree in the 20° house showed any symptoms of the disease, and no symptoms appeared at 24 or 28°. All of the trees of this experiment were placed out of doors during the summer and early winter of 1942 and returned in December to the same greenhouses they had occupied in the spring. Those in the 16° house showed typical leaf symptoms of yellows by March 17, 1943, whereas none of those at the higher temperatures showed symptoms of the disease during the 1943 greenhouse season.

In another experiment in the spring of 1942, potted Montmorency trees in the greenhouse were budded from yellows sources, carried through the summer and early winter out of doors, and then brought into the greenhouses for tests on the expression of leaf symptoms. Four such trees were placed at each of the following temperatures: 16, 20, 24, and 28° C. Typical leaf symptoms of yellows developed freely on all 4 trees in the 16° house in the spring of 1943. No symptoms appeared on the trees incubated at the higher temperatures.

On January 18, 1943, dormant buds from a Montmorency tree affected by yellows were inserted in dormant Montmorency nursery trees that had recently been potted in the greenhouse. Three of these budded trees were incubated at each of the following greenhouse temperatures: 16, 20, 24, and 28° C. On March 17, two of the trees at 16°, and none of the others, showed leaf symptoms of yellows. None of the other trees developed symptoms of yellows during the greenhouse season of 1943.

Because of the indications in the preliminary experiment of 1942 that 16° C. is a favorable temperature for development of the leaf symptoms of yellows, a greenhouse in which most of the experimental potted trees were carried in 1943 was held at comparatively low temperature. At night the temperature varied from 12 to 16°, but in the middle of the day it often ran in the range of 24 to 28° C. On scores of sour cherry trees incubated in this greenhouse, the typical leaf symptoms of yellows developed freely.

The results thus far available indicate clearly that 16° C. is a favorable temperature for expression of the leaf symptoms of cherry yellows and that

these symptoms are masked at approximately constant temperatures of about 20° C. or higher. Closer studies of the effects of diurnal fluctuations in temperature and correlations with epidemiological data remain to be made. In the meanwhile, the use of potted plants at suitably controlled temperatures in the greenhouse affords favorable opportunity for experimentation on many aspects of cherry yellows and on other virus diseases with which it may be associated.—G. W. KEITT and J. DUAIN MOORE, University of Wisconsin, Madison, Wisconsin.

*A New Mosaic Disease of Beans.*—In 1939, Michelite pea beans in two fields near Batavia, New York, were observed, infected with a mosaic virus producing symptoms that, to all appearances, were identical with those of the common bean mosaic. The incidence of the disease was so high that the two fields were not harvested. This was the first time, as far as the writers are aware, that the Michelite had been grown in New York State, and the fact that it was susceptible to the mosaic virus was surprising, since the variety[1] had been reported to be resistant. Also, during the last few years many complaints have been received from growers stating that the Robust, the outstanding pea bean for 25 years, was beginning to exhibit large percentages of plants showing mosaic symptoms. The Robust bean, shortly after its introduction, had been proved immune from the virus[2] and had remained so for years. Whether or not the occurrence of this disease in the Robust was due to impurities in the stock could not be determined from observation.

In 1942 a number of samples of both Robust and Michelite beans were obtained from as reliable sources as possible. Tests with an isolate of mosaic virus, obtained from a plant grown in New York State, demonstrated that these two varieties were 100 per cent susceptible to the virus isolate used. Later, 6 isolates of mosaic virus taken from diseased seedlings, 3 of which the seed had been grown in New York State, 1 in California, 1 in Idaho and 1 in the Canal Zone, were used in inoculation experiments. These isolates were maintained on Red Kidney beans that previously had been shown free from mosaic. The Robust and Michelite proved susceptible to 2 isolates of the virus from New York seed, but immune from the other 4 virus isolates.

These results clearly demonstrate that there are 2 strains of the bean-mosaic virus or, at least, that there are two virus entities producing a mosaic disease on this plant. The symptoms produced by both are essentially those described for the common bean mosaic, and both viruses are seed-transmitted. Further experiments proved that the Red Kidney and the Bountiful are susceptible to the 2 viruses; that the Norida, Red Mexican 3 (Univ. Idaho), and Great Northern 15 (Univ. Idaho), together with the Robust and Michelite, are susceptible to what might be called the new strain of the

[1] Down, E. E., and J. W. Thayer. The Michelite bean. Mich. Agr. Exp. Stat. Sp. Bull. 295. 23 pp. 1938.

[2] Reddick, Donald, and Vern B. Stewart. Varieties of beans susceptible to mosaic. Phytopath. 8: 530–534. 1918.

virus, but not to the old; and that the Great Northern 1[3] and 59 (Univ. Idaho), Ashley's wax (U.S.D.A.), Cooper's wax (U.S.D.A.), and Refugee 5 (U.S.D.A.) are immune from both strains. The fact that in certain bean varieties there exists immunity from the virus that is infecting the pea beans is hopeful. Breeding work is underway to combine immunity from the 2 viruses in a small white type of bean of desirable characters.—B. LORIN RICHARDS and WALTER H. BURKHOLDER, Department of Plant Pathology, Cornell University, Ithaca, New York.

*A Foliar Mottle and Necrosis in Chippewa Potatoes Associated with Infection by a Strain of the Potato X Virus.*—The rather obscure tuber-perpetuated mosaic disease described in this note could not be definitely identified with any known potato virus. It was first noticed in a few tuber units of Wisconsin-grown Chippewa potatoes in a southern test planting in 1939 and again in 1941. In 1942 and 1943 additional Chippewa specimens were obtained from a large seed planting in northern Wisconsin and in an early table-stock planting of the same seed in the central area. Although the symptoms in the field bear some resemblance to early symptoms of mild mosaic on varieties such as Cobbler and Triumph, they are easily distinguished from it by characteristic irregular chlorotic mottle patches in the intercostal areas of the upper leaves of young plants and by the small scattered necrotic flecks on the older leaves. Later the irregular flecking is manifest to a slight extent in the upper leaves. Foliage symptoms vary in severity under both field and greenhouse conditions but are within a well-defined pattern range, and infected plants can be easily recognized. Lethal acronecrosis has not been observed under any conditions. External and internal stem and petiole tissues of affected plants are normal, and cells of the xylem, phloem, and cortex show no discoloration. Tubers are slightly smaller than normal but show no manifestations of infection.

Repeated aphid (*Myzus persicae* Sulz. and *Macrosiphum solanifolii* Ash.) transmission tests yielded negative results. When the virus was transmitted mechanically to Chippewa, Sebago, Triumph, Red Warba, and Russet Burbank varieties and to first generation potato seedlings derived from seed of Katahdin by selfing, the first sign of infection appearing on the inoculated leaves in 12 to 15 days as small, irregular, scattered, necrotic lesions, which penetrated the thickness of the leaf. The early recognizable systemic symptoms appeared as a slight, interveinal, chlorotic mottle of the younger leaves followed by necrotic flecking of the interveinal areas, which remained as isolated areas, showing no tendency to coalesce. Seedling 41956 is immune.

Artificially inoculated *Nicotiana tabacum* L. (var. Connecticut Havana No. 38) developed especially clear, small, light-colored, concentric ring-type lesions on the surface of inoculated leaves followed by systemic symptoms consisting of discrete ring-type and irregular line- and ring-patterns. There

[3] This variety was received from R. A. Emerson. The writers do not know whether it is of Idaho or U.S.D.A. origin.

is no evidence of vein chlorosis, vein-banding or mottle in the systemically affected tobacco plants. The ring-spot type of lesion is displayed to best advantage on *N. rustica*. Tobacco plants, previously infected with a mottle strain of potato X virus, did not show the above symptoms when reinoculated with the virus from diseased Chippewa plants, indicating strongly that the Chippewa virus is a variant of virus X. It would appear, therefore, that this variety, which is highly resistant to mild mosaic and is a masked carrier of common strains of potato X, is subject to infection by an uncommon strain of the latter, which causes mosaic mottle not unlike that of mild mosaic on Cobbler and Triumph. In preliminary temperature studies, symptoms developed in affected Chippewa plants more rapidly and severely at 16° to 20° than at 22° to 24° C., while they were entirely suppressed at 28° C.—R. H. LARSON, Wisconsin Agricultural Experiment Station, Department of Plant Pathology, University of Wisconsin, Madison, Wis.